University of Bath

PHD

Presynaptic receptor - mediated facilitation of vascular adrenergic neurotransmission in the rat

Meghji, Samoon Amiralli

Award date:
1988

Awarding institution:
University of Bath

Link to publication

Alternative formats
If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

Copyright of this thesis rests with the author. Access is subject to the above licence, if given. If no licence is specified above, original content in this thesis is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/). Any third-party copyright material present remains the property of its respective owner(s) and is licensed under its existing terms.

Take down policy
If you consider content within Bath's Research Portal to be in breach of UK law, please contact: openaccess@bath.ac.uk with the details. Your claim will be investigated and, where appropriate, the item will be removed from public view as soon as possible.

Download date: 05. May. 2023

PRESYNAPTIC RECEPTOR - MEDIATED FACILITATION OF VASCULAR ADRENERGIC NEUROTRANSMISSION IN THE RAT

Submitted by Samoon Amiralli Meghji

for the degree of Ph.D.

of the University of Bath

1988

COPYRIGHT

"Attention is drawn to the fact that copyright of this thesis rests with its author. This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the prior written consent of the author".

"This thesis may be made available for consultation within the University Library and may be photocopied or lent to other libraries for the purposes of consultation".

UMI Number: U498611

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

UMI U498611
Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.
All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

UNIVERSITY OF BATH
LIBRARY
14 SEP 1988
PHD
5022949

Summary

The aim of this project was to show the presence of enhanced presynaptic receptor-mediated mechanisms facilitating vascular adrenergic neurotransmission in preparations from genetically hypertensive rats (spontaneously hypertensive, SH, rat and New Zealand Hypertensive, NZH rat) compared to that in normotensive rats. Isoprenaline and angiotensin II (AII) potentiated the pressor response to peri arterial nerve stimulation (PNS) in the isolated perfused mesenteric vasculature and in the isolated perfused kidney. This potentiation was found to be enhanced in preparations from the hypertensive rats compared to that in preparations from normotensive rats (Wistar and New Zealand Normotensive, NZN, rats). ICI 118,551, but not atenolol, effectively inhibited the isoprenaline induced facilitation of vascular adrenergic neurotransmission without having any effect on the AII induced potentiation of the pressor responses to PNS and noradrenaline (NA) infusion.

That the facilitatory mechanisms are located presynaptically was inferred from the comparison of the effects of isoprenaline on the pressor response to PNS and exogenous NA. Both, [Sar^1-Ile^8] angiotensin II and captopril effectively inhibited the isoprenaline induced facilitation of vascular adrenergic neurotransmission, thereby showing that isoprenaline probably activated the vascular renin-angiotensin system to generate AII. The

locally generated AII then facilitates vascular adrenergic neurotransmission and this effect was found to be enhanced in preparations from hypertensive rats compared to that in the normotensive rats. The next series of experiments were conducted to see if the same mechanisms were present in experimentally induced models of hypertension.

Two models of experimental hypertension were looked at; the Goldblatt, two-kidney, one-clip renal hypertensive rat and oestrogen-induced hypertension. To this effect it was found that the presynaptic β_2-adrenoreceptor-mediated facilitation of vascular adrenergic neurotransmission was enhanced in the experimental models of hypertension compared to control normotensive rats. However, the enhanced presynaptic AII-receptor-mediated facilitation of vascular adrenergic neurotransmission was only found in preparations from the renal hypertensive rat.

Plasma adrenaline and noradrenaline levels were elevated in all models of hypertension compared to that in normotensive rats.

It therefore seems that sympatho-adrenal activity plays an important role in the development and maintenance of hypertension.

It yet remains to be seen as to what causes the onset of genetic hypertension.

To my parents, Ameeralli and Shireen

Acknowledgements

I would like to express my gratitude to my academic supervisors, Dr. P.H. Redfern, and Mr. A.J. Draper, for all the help and guidance over the last few years.

I would also like to thank the members of staff of the Animal House, Bath University for all the help they have given me and to Mrs. J. Curtis for her assistance in typing this thesis.

Finally, I wish to thank my wife, Khadija, for her continued support and encouragement over the last few years.

Contents

CHAPTER 1

INTRODUCTION

This thesis is concerned with the enhanced facilitation of adrenergic neurotransmission in isolated perfused vascular preparations from genetically hypertensive rats and experimentally hypertensive rats compared to that in normotensive rats.

The hypothesis that adrenergic nerve terminals possess β-adrenoreceptors which facilitate transmitter release is relatively new (Adler-Graschinsky and Langer, 1975; Stjarne and Brundin, 1975; Hedqvist and Moawad, 1975) but, as so often happens with hindsight earlier observations pointing that way can be found. Experimentally the hypothesis is based on the ability of β-adrenoreceptor agonists to increase and of β-adrenoreceptor antagonists to decrease the amount of transmitter released by nerve stimulation. In early experiments on the depression by catecholamines of transmission through autonomic ganglia it was noted that in low concentrations adrenaline and especially isoprenaline facilitated rather than depressed transmission (Matthews, 1956; Pardo et al 1963). A similar facilitatory effect of isoprenaline on the release of a transmitter, this time acetylcholine from field-stimulated guinea-pig longitudinal muscle preparation, was reported previously (Paton and Vizi, 1969).

The evidence that β-adrenoreceptor antagonists could depress responses to adrenergic nerve stimulation, even in tissues where the postsynaptic receptors were alpha -adrenoreceptors, was particularly clear; in the first

report (Day et al, 1968) propranolol was shown to be as effective as guanethidine in reducing the response to sympathetic nerve stimulation in the rat vas-deferens, the rabbit ileum and the perfused rabbit ear artery. Like guanethidine, propranolol potentiated the response to noradrenaline (NA). The effect did not seem to be caused by the known local anaesthetic action of propranolol since a ten-fold higher concentration of lignocaine was ineffective. Lignocaine and propranolol are about equiefficative as local anaesthetics (Morales-Aquilera and Williams, 1965). This ability of propranolol to block nerve responses was confirmed and extended to other similar drugs and other tissues such as the guinea-pig vas deferens (Mylecharane and Raper, 1970), the cat nictitating membrane (Eliash and Weinstock, 1971) and spinal vasoconstrictor nerves (Ablad et al, 1970). Propranolol for a time was regarded as an adrenergic neurone blocker, though its action was mediated through β-adrenoreceptors, since its neurone blocking activity did not correlate with postsynaptic β-adrenoreceptor blockade (Mylecharane et al, 1970, Eliash et al, 1971).

Adler-Graschinsky and Langer (1975) first suggested that the release of NA from sympathetic nerve endings might be modulated by presynaptic β-adrenoreceptors through a positive feedback mechanism. They used isolated guinea-pig atria and demonstrated that isoprenaline increased the stimulation-induced overflow of NA whereas propranolol decreased this overflow. A facilitatory effect of

isoprenaline on stimulation-induced overflow of NA has been shown in other tissues: in cat aorta and nictitating membrane (Langer et al, 1975); rat cerebral cortex (Farnebo and Hamberger, 1974); guinea-pig atria (Rand et al, 1976); human omental arteries and veins (Stjarne, 1975; Stjarne and Brundin, 1976).

Nerve impulses in most sympathetic nerves elicit a response in the effector cell (e.g. smooth muscle) by releasing the chemical transmitter NA, which affects specific receptor sites on the effector cells (α- or β-adrenoreceptors). The amount of NA released, and therefore the degree of response elicited, is ultimately under the control of the central nervous system, which controls the amount and rate of delivery of nerve impulses. However, there is now a substantial body of evidence suggesting that there are peripheral receptor-mediated mechanisms which can modulate the amount of NA released by each nerve impulse.

Inhibition of the induced release of endogenous NA has been observed with NA and other α-adrenoreceptor agonists. In addition, acetylcholine, choline, dopamine, prostaglandin E_2, adenosine, histamine and 5-hydroxytryptamine have also been shown to inhibit the release of endogenous NA (Westfall, 1977, Starke, 1977). On the other hand there have been reports of enhanced release of stimulation-induced NA by agents that activate angiotensin II (AII) receptors (Kawasaki et al 1982b),

β-adrenoreceptor agonists (Stjarne and Brundin, 1975; (Kawasaki et al, 1982a, 1984) and nicotinic agonists (Westfall and Brasted, 1974).

The experiments with β-adrenoreceptor agonists have given rise to the hypothesis suggesting that the release of NA may be subject to a positive feedback modulation in addition to the well-established presynaptic α-adrenoreceptor-mediated negative feedback mechanism (Starke, 1974b, 1975b). According to the hypothesis, the positive feedback mechanism would be activated by low concentrations of the transmitter, NA, leading to an increase in the release of the transmitter per impulse. As the concentration of the NA in the synapse increases, the positive feedback would then be replaced by the negative feedback process mediated by the presynaptic α-adrenoreceptor (Adler-Graschinsky et al, 1975). The terms presynaptic, or prejunctional, have been coined to indicate that these release-modulating receptors are located before the neuro-effector junction (Starke and Langer, 1980), that is to say, on or next to the terminal varicosities of the post ganglionic nerve ending. However, exogenous NA has only been shown to increase transmitter NA release when presynaptic α-adrenoreceptors are blocked. An alternative hypothesis (Stjarne and Brundin, 1976) suggests that the physiological role of presynaptic β-adrenoreceptors may be to detect circulating adrenaline (Ad) from the adrenal medulla rather than NA from sympathetic nerve terminals, during

conditions of stress, Ad released from the adrenal medulla would thus increase sympathetic nerve activity. Furthermore, it has been demonstrated, in isolated guinea-pig atria, that β-adrenoreceptor antagonists consistently reduced transmitter NA release when Ad was incorporated into the transmitter stores along with NA, but failed to do so when only NA was present (Rand et al, 1979). This suggests that transmitter NA does not facilitate its own release by activating a positive feedback loop through presynaptic B-adrenoreceptors; however, if adrenaline is also present, the facilitatory mechanism is activated. It is conceivable that Ad, taken up from the circulation and incorporated into transmitter stores of noradrenergic axons, is released on stimulation along with NA and activates the positive feedback mechanism through presynaptic β-adrenoreceptors.

Adrenaline can be released as a co-transmitter with NA from sympathetic nerves for some time after its initial uptake from the circulation since Ad taken up into sympathetic nerves has a longer half-life (approximately four hours) than in the blood (approximately one minute) (Majewski et al, 1981a). Ad facilitation of NA release has been shown in the anaesthetized rabbit (Majewski et al, 1982a) where Ad administration caused an increase in the rate of NA release into the plasma. This occurred after the Ad had disappeared from the plasma, but at a time when the Ad levels in sympathetically-innervated tissues were raised. Pretreatment of the rabbits with

desipramine to block neuronal uptake, or propranolol to block β-adrenoreceptors, prevented the facilitatory effect of Ad. This suggests that the neuronally-released adrenaline can activate presynaptic β-adrenoreceptors to facilitate NA release in vivo. Indeed, it has been demonstrated that Ad is a hundred times more potent than NA in enhancing the release of NA from sympathetic nerves (Dahlof et al, 1978). Thus, it seems that Ad rather than NA may be of physiological importance in the activation of this positive feedback mechanism.

Lands et al (1967) proposed that β-adrenoreceptors could be divided into two sub types, β_1- or β_2-adrenoreceptors, according to the rank order of potency of a series of agonists. Attempts have been made to characterize the presynaptic β-adrenoreceptors in terms of these postsynaptic β-adrenoreceptor subtypes. Stjarne and Brundin (1976), using relatively selective β_1- and β_2-adrenoreceptor agonists and antagonists, concluded that in human omental arteries and veins the presynaptic receptors were of the β_2-subtype. Similar results have been obtained in the rat portal vein (Dahlof et al, 1978 ; Westfall et al, 1979).

However, not all experiments have yielded clear results. In the guinea-pig atria, neuronally-released Ad but not neuronally-released NA facilitated the stimulation-induced release of NA, suggesting that the presynaptic β-adrenoreceptors are of the β_2-subtype. Similarly in rat

isolated atria in the presence of phenoxybenzamine, Ad, but not NA, enhanced the stimulation-induced efflux of NA. Again, this indicates the presence of β_2-adrenoreceptors, however metoprolol reduced the facilitatory effect of Ad (Majewski et al, 1981b).

Further, in human digital arteries the β_2-adrenoreceptor agonist, salbutamol, was more potent than the selective B_1-adrenoreceptor agonist, dobutamine, in enhancing the stimulation-induced efflux of NA (Stevens et al, 1982).In the isolated mesenteric vasculature it has been shown that butoxamine, a selective β_2-adrenoreceptor antagonist, reduced the isoprenaline-induced potentiation of the pressor response to periarterial nerve stimulation (Kawasaki et al, 1982a).

On the other hand Majewski et al (1981b, 1982b) have shown that metoprolol reduces the increase in blood pressure due to adrenaline implants in normotensive rats, thereby indicating that the presynaptic B-adrenoreceptor was of the β_1-subtype. Work done previously in the guinea-pig pulmonary arteries showed that l-metoprolol and not, d-metoprolol, inhibited isoprenaline-induced increases in ^3H efflux by transmural field stimulation (Misu et al, 1983). Metoprolol is characterised as a relatively selective β_1-adrenoreceptor antagonist and these results therefore suggest that there may exist additional presynaptic β_1-adrenoreceptors in the guinea-pig atria (Misu et al, 1983). Further experiments

conducted by Misu et al (1983) show that acebutolol, bevantolol and practolol did not antagonize, whereas metoprolol, butoxamine and H35/25 antagonised salbutamol-induced increases in impulse evoked ^{3}H efflux supporting the existence of presynaptic β_2-adrenoreceptors rather than of the β_1- subtype adrenoreceptor. There is also a possibility that presynaptic β-adrenoreceptors might differ from postsynaptic classical β-adrenoreceptors, probably in the same way as the presynaptic and postsynaptic α-adrenoreceptors differ in their affinity for agonists and for antagonists (Langer, 1980). Indeed, in terms of potency in blocking β_2-adrenoreceptors, metoprolol is slightly more potent than the β_2-adrenoreceptor antagonist butoxamine (Minneman, Hedberg and Molinoff, 1979). Work done on pA_2 values of β-adrenoreceptor antagonists (Harms, 1976) showed that in certain situations metoprolol probably behaves as a non-selective antagonist on postsynaptic β-adrenoreceptors as do propranolol and pindolol.

Therefore, the results using agonists in all cases suggest that the presynaptic β-adrenoreceptor is of the β_2-subtype but the results with the β-adrenoreceptor antagonists reveal no clear picture; this perhaps relates either to the lack of selectivity of these agents or to mixed receptor subtypes or to different receptor characteristics compared to postsynaptic β-adrenoreceptors.

That facilitatory presynaptic β-adrenoreceptors play a role in human essential hypertension has been an attractive hypothesis considering the important place the β-adrenoreceptor antagonists have in antihypertensive therapy. It has often been stated that at least part of the antihypertensive effect of this group of drugs results from a sympatho-inhibitory effect caused by blockade of presynaptic β-adrenoreceptors (Adler-Graschinsky, 1975; Majewski et al, 1981b). This hypothesis presumes that presynaptic β-adrenoreceptors are activated by some physiological agonist, and Ad seems to be the prime candidate for this role considering its effects on presynaptic β-adrenoreceptors.

One test of this hypothesis is whether high blood pressure develops when the Ad input into the circulation is chronically or intermittently increased. Studies in rats where Ad was given as a slow release preparation (Majewski et al, 1981b), incorporated into osmotic mini pumps (Majewski et al, 1982b; Borkowski and Quinn, 1985), showed that sustained increases in blood pressure did develop and these increases were prevented by concomitant treatment with a β-adrenoreceptor antagonist.

The Spontaneously Hypertensive (SH) rat (Okamoto and Aoki, 1963) has been widely used as a model for studying human essential hypertension (Grollman, 1972). However the actual mechanisms underlying the development and/or maintenance of hypertension in the SH rat remains

uncertain. The results from an investigation carried out by Mulvany on the resistance vessel abnormalities in the SH rat (Mulvany, 1984) indicate that the heart/body weight ratio and the media/lumen ratio of the resistance vessels are increased in the twelve to fourteen week old SH rat compared to age matched normotensive rats. It is suggested that this is due primarily to genetic factors rather than to the increased blood pressure, and these structural abnormalities do not appear to be a sufficient cause of the increased blood pressure. The calcium sensitivity of resistance vessels, which is also increased in the SH rat, may be associated with the genetic factors responsible for the increased blood pressure (Mulvany, 1984).

Another hypothesis postulates an increased total vascular resistance resulting from enhanced sympathetic adrenergic nerve tone as the cause of increased blood pressure in the SH rat (Iriuchima, 1973; Hallbeck and Folkow, 1974; Judy et al, 1976; Nagatsu et al, 1976; Coote and Sato, 1977; Cheng and Shibata, 1980). This increased adrenergic tone in the SH rat may be due in part to an increased facilitatory and/or a decreased inhibitory modulation of adrenergic neurotransmission.

Indeed, previous reports (Kawasaki et al, 1982a) have indicated that in the isolated mesenteric vasculature of the SH rat an increased modulation of the facilitatory presynaptic β-adrenoreceptor exits. This indicates that

the increased adrenergic tone in the SH rat may be due in part to enhanced facilitatory presynaptic β-adrenoreceptor-mediated modulation of the adrenergic neurotransmission.

Basal plasma Ad and NA levels in the SH rat are not different compared to normotensive Wistar-Kyoto rats but stress-induced release of Ad and NA into the circulation is significantly higher in the SH rat compared to that in the Wistar-Kyoto rat (McCarty et al, 1978). This increase in plasma Ad could, it is postulated, activate the presynaptic β-adrenoreceptor and thereby cause an increased adrenergic vascular tone in the SH rat. However, it has been shown that propranolol has an acute antihypertensive effect in the absence of circulating Ad in bilaterally adrenalectomised Wistar-Kyoto rats made hypertensive with methylprednisolone (Burris et al, 1984). The results of the work carried out by Burris et al (1984) indicate that propranolol reduces blood pressure at least in part by mechanisms other than presynaptic β-adrenoreceptor blockade. There is evidence for a central site of action of propranolol (Alexander et al, 1975; Garvey et al, 1975; Day et al, 1973; Reid et al, 1974) and this could account for the propranolol-induced reduction of central sympathetic activity. Finch and Leach (Finch et al, 1970) have shown that the development of renal and DOCA/NaCl hypertension in the rat was unaffected by demedullation and that chemical sympathectomy using 6-hydroxydopamine followed by

demedullation also failed to prevent the development of these types of hypertension. The results of the work of Finch et al (1970) suggest that experimental hypertension in the rat is independent of the adrenal medulla provided an adequate salt intake is maintained.

However, bilateral adrenal demedullation of young SH rats has been shown to attenuate the development of hypertension and to reduce significantly vascular responses to sympathetic nerve stimulation (Borkowski and Quinn, 1983).

Besides the involvement of adrenergic and adrenal medulla in the development and/or maintenance of hypertension described above another mechanism may contribute to the increased vascular tone, and therefore result in elevated vascular resistance, in SH rats and in other animal models of hypertension, and this is the renin-angiotensin system. The role of the renin-angiotensin system in blood pressure maintenance is unsettled. Studies with radioactive angiotensin II (AII) indicate that this octapeptide is bound to specific receptor sites in vascular smooth muscle (Baudoin et al, 1971). This suggests that renin, through angiotensin, has a specific function in this tissue (rabbit aorta). However, in chronic renal hypertension and in spontaneous hypertension in rats (Okamoto and Aoki, 1963) the relationship of renin to elevated blood pressure has been questioned because of the observation that circulating renin is normal or low compared to

normotensive animals (Koletsky et al, 1967; Vincent et al, 1976). On the other hand, others (deJong et al, 1972) have reported that renin increases with age in SH rats.

Renin is known to be present in the blood vessel walls of pigs and rats (Dengler, 1956; Rosenthal et al, 1969) and thus might play a role in blood pressure maintenance. Injection of anti renin into dogs with either acute renin-induced hypertension or chronic one-kidney Goldblatt renal hypertension caused a rapid, partial drop in blood pressure during the first twenty minutes after infusion in the first model and a much slower drop (4 to 10 days) in the second. The rate of production of the hypotensive effect is consistent with the hypothesis that neutralization of circulating renin occurred in the acutely hypertensive animals and of vascular wall renin in chronically hypertensive ones (Hill et al, 1970).

In the rat, the action of an angiotensin II antagonist, which can penetrate the arterial wall and angiotensin II antiserum, which cannot, suggested that renin generates angiotensin at a local vascular level at a site not readily accessible to antiserum (Thurston and Swales, 1974). Additional support for this theory is that converting enzyme inhibitor causes a fall in blood pressure for several hours after bilateral nephrectomy, indicating that the renin-angiotensin system maintains blood pressure in this model even after plasma renin has fallen to insignificant levels (Thurston et al, 1977). In

addition the pressor action of renin is present in nephrectomised animals without detectable circulating renin (Schaechtelin et al, 1964). Considerable evidence implicates the sympathetic nervous system and the catecholamines in the regulation of renin secretion (Vander, 1965; Gordon et al, 1967). In view of the close relation of adrenergic nerve endings to juxtaglomerular cells (Wagermark et al, 1968) and the release of renin from renal cortical cell suspension (Michelakis et al, 1969), a direct effect of adrenergic hormones on renin secretion seems likely. The suggestion that renin secretion provoked by catecholamine infusion or sympathetic nerve stimulation is mediated by β-adrenoreceptors receives support from studies in dogs (Ganong, 1972; Tanigawa et al, 1972) and man (Michelakis et al, 1972), using adrenoreceptor antagonists.

There is evidence that all the components of the renin-angiotensin system are present in the mesenteric vasculature of the rat (Desjardins-Giasson et al, 1981). In addition, there have been reports that utilization of renin substrate within the mesenteric vascular wall of rats by renin, or renin-like enzymes, resulted in the formation of angiotensin I, which was then converted to angiotensin II. The locally-generated angiotensin II was shown to enhance the vasoconstrictor responses to adrenergic nerve stimulation of the mesenteric vasculature (Malik et al, 1976).

Supporting the suggestion that increased sympathetic adrenergic tone in the vasculature of the SH rat, postulated to be the aetiology of this hypertension (Iriuchijuma, 1973; Judy et al, 1976; Cheng and Shibata, 1980), may be due to an increased facilitatory and/or decreased inhibitory modulation of vascular adrenergic neurotransmission, are the reports that the facilitatory modulation of vascular adrenergic neurotransmission mediated by presynaptic AII receptors were enhanced in the isolated perfused mesenteric vascular preparation from SH rats (Eikenburg et al, 1981; Kawasaki et al, 1982b; Clough et al, 1982) and also in the in situ, blood-perfused mesentery of SH rats (Cline, 1982; 1983). A variety of changes in other modulatory mechanisms in several vascular beds has been reported to occur in the SH rat, with most of these reported changes supporting increased release of NA (Kamikawa et al, 1980; Lokhandwala and Eikenburg, 1983).

It is well documented that a β-adrenoreceptor agonist, such as isoprenaline, can stimulate the release of renin from the kidney and elevate plasma renin activity in vivo (Keeton and Campbell, 1980). This in turn would lead to the production of AII.

The aims of this thesis are:-

i) To determine whether in the SH rat and the New Zealand Hypertensive (NZH) rat (Phelan, 1968) there exists an enhanced presynaptic β-adrenoreceptor-mediated facilitation of adrenergic neurotransmission in the vascular preparations compared to that in normotensive Wistar rats and New Zealand Normotensive (NZN) rats.

ii) To investigate if facilitatory presynaptic β-adrenoreceptors are present in the rats, the nature of the β-adrenoreceptor i.e. whether it is of the β_1- or β_2-subtype.

iii) Furthermore, the involvement of the vascular renin-angiotensin system in β-adrenergic receptor-mediated facilitation of vascular neurotransmission in rats is investigated, especially in regard to enhanced angiotensin II-receptor-mediated facilitation of adrenergic neurotransmission in the vasculature of the hypertensive rat

iv) The involvement of the above two presynaptic-receptor-mediated effects on the modulation of adrenergic neurotransmission in vascular preparations from experimentally-induced

hypertensive Wistar rats is also investigated. Two methods of inducing experimental hypertension in the Wistar rats were employed. They were:-

a) Mechanical occlusion of the renal artery to induce renal hypertension; the "two-kidney, one-clip renal hypertensive" rat (Goldblatt et al, 1934), and

b) Chemically-induced hypertension by treating the Wistar rats chronically with ethinyloestradiol.

The ultimate aim of this thesis is to show if facilitatory presynaptic β-adrenoreceptor and presynaptic AII receptor are enhanced in hypertensive rats and that this enhancement is in part responsible for the increased vascular tone in the animals which is indicated in the development and/or maintenance of the hypertension.

In order to investigate the subtype of the β-adrenoreceptors, a selective β_2-adrenoreceptor antagonist, ICI 118,551, was employed in the experiments. Previously described β_2-selective antagonists such as butoxamine, H35/25 and IPS 339 are lacking in potency, specificity, or appropriate β_2-selectivity. ICI 118,551 possess a high degree of selectivity and specificity for the β_2-adrenoreceptor (Bilski et al, 1983). The β_2/β_1-

selectivity ratios, in vitro, are 123 for ICI 118,551 and 2.2 for propranolol (Bilski et al, 1983). ICI 118,551 has no partial agonist activity but has a membrane-stabilising action similar to that of propranolol (Bilski et al, 1983). Since in the Goldblatt, two-kidney, one-clip renal hypertensive rat the level of circulating renin is elevated and is accompanied by an increased concentration of renin in the arterial walls (Garst et al, 1978) and since the sympathetic nervous system is also implicated in this type of hypertension (Laverty et al, 1961; McCubbin et al, 1963), the effect of ICI 118,551 on the induced hypertension was also investigated.

Plasma levels of Ad and NA were also monitored in SH rats, Wistar rats and all the forms of experimentally-induced hypertensive rats.

In the next five chapters, the methods and results of various investigations are shown followed by a short discussion of the results at the end of each relevant chapter. A fuller and more concise discussion is in the "Discussions" chapter (Chapter 7)

CHAPTER 2

ISOLATED PERFUSED MESENTERIC VASCULATURE

2.1 Preliminary work

The isolated mesenteric vasculature was set up as described below and perfused with oxygenated Krebs. Ideal parameters for peri-arterial nerve stimulation (PNS) in order to give consistent and repeatable pressor responses were found to be 30Hz, 80V, 1msec pulse width for 10 seconds. Unlike Kawasaki et al (Kawasaki et al, 1982a, 1982b, 1984), I found that if too low a frequency was used, i.e. below 20Hz, and duration of the stimulation period increased to over 15 seconds, the preparation would not respond consistently to the PNS and the preparation would not last for more than 20 to 30 minutes. Kawasaki et al, 1982a, 1892b, 1984) used 10Hz for 30 seconds giving a total of 300 impulses. Therefore the total number of impulses delivered to the tissue per PNS period was similar in both our protocols.

Next, a concentration of NA necessary to give an increase in perfusion pressure of the preparation roughly similar to that caused by PNS (30Hz, 80V, 1msec, 10sec) was determined; this concentration was found to be 0.1ml of a 10^{-6}M solution of noradrenaline bitartarate.

2.2 Methods

2.2i Isolated perfused mesenteric vascular bed

The rats were killed by first stunning and then dislocating the cervical vertebrae. The isolated mesenteric vascular bed was prepared essentially by the method of McGregor (McGregor, 1965). Briefly a ventral midline incision was made through the skin and body wall from the lower abdomen to the xiphisternum. The intestines were reflected to the left and the superior mesenteric artery was freed approximately 1 cm. distal to its bifurcation from the aorta and ligated. A portex 16 gauge (3 FG) polyethylene cannula, outer diameter 1.02 mm, was inserted into the superior mesenteric artery and the blood was gently flushed out with 10 ml of the Kreb's solution. The pancreaticoduodenal, ileocolic, colic and caecal branches of the superior mesentery artery were tied off as were all branches of the latter supplying the ileum except the four most distal divisions. The mesenteric vascular bed was freed from the intestines by cutting close to the border with the intestines. During the dissection, care was taken to keep the mesenteric vasculature moist with oxygenated Kreb's. The isolated vasculature was placed in a 5 ml water-jacketed organ bath maintained at 37°C and perfused with a modified Kreb's solution at a constant flow rate of 5 ml/min by means of a Watson Marlow peristaltic pump. The modified Kreb's was of the following composition (mM):

Sodium Chloride,	120;
Glucose,	11;
Sodium bicarbonate,	25;
Magnesium Sulphate (heptahydrate),	1.2;
Potassium Chloride,	4;
Calcium Chloride,	2.5;
Ascorbic acid,	0.289;
Sodium edetate,	0.027;

pH = 7.4

The perfusing solution was aerated with a mixture of 95% oxygen and 5% carbon-dioxide before passing through a warming coil maintained at 38°C. Changes in perfusion pressure were measured at a point close to the cannula by means of a pressure transducer (Bell and Howell, type 4-422-0001) and recorded on a Devices (M2) recorder.

After allowing time for the basal perfusion pressure to stabilize, usually 15 min, the perfused mesenteric vasculature was subjected either to periarterial nerve stimulation (PNS) or to a bolus of noradrenaline (NA). The PNS was delivered at 5 min. intervals via bipolar platinum electrodes placed around the superior mesenteric artery. Supramaximal rectangular pulses, 1 msec, 80V, were applied for 10 sec. at 30Hz by means of a Grass, model S44, stimulator. The neural basis of the pressor response mediated by stimulation of the arterial adrenergic nerve was confirmed in the preparations from SHR male and female, NZH female and control animals by abolition of the response after perfusion with

guanethidine (0.01mM, n=3 animals for each group). Noradrenaline (0.1 ml of 10^{-6}M soln) was injected directly into the perfusate proximal to the arterial cannula at 5 minute intervals.

Once stable responses to PNS or NA infusion had been demonstrated, perfusion with the other drugs began. Perfusion of B-adrenoceptor agonists and angiotensin II (AII) was begun 3 min. before and throughout PNS or NA infusion. In the experiments involving beta-adrenoceptor antagonists the perfusion of the antagonist was begun 10 minutes before simultaneous perfusion with the agonist. Infusion of the angiotensin II receptor antagonist [Sar^1-Ile^8] angiotensin II (Sar) was started 2 minutes before simultaneous perfusion with angiotensin II. I found like Kawasaki et al (1982b; 1984), that the second response elicited by PNS when perfusing B- adrenergic receptor agonist was greater than the first i.e. the response to PNS at 8 minutes from the start of perfusion was greater than that at 3 minutes Therefore all the data were derived from the second response to PNS or NA infusions during perfusion of the drugs. The exception to this was data of responses to PNS or NA infusion from experiments involving cocaine. Only one response, at the 8th minute, was taken when cocaine was perfused. This was because during PNS the first response was much greater than the second. This could be explained by the fact that cocaine caused blockade of the uptake mechanism, therefore causing most of the neurally-released noradrenaline to be washed

away by the perfusing solution. In the experiments involving antagonists, the response to PNS or NA infusion before the perfusion of the antagonist was taken as control.

PNS and NA response data after drug perfusion in the preparations are expressed as a percentage of the control pressor response in order to standardize the data.

Male and female Japanese Hypertensive (SH) rats (Okamoto and Aoki, 1963) and female New Zealand Hypertensive rats (NZH) (Phelan, E.L., 1968) were compared to age matched male and female Wistar rats (University of Bath strain) and female New Zealand Normotensive rats (NZN) (University of Bath strain), respectively. The animals used were 14-16 weeks old and were supplied by the University of Bath Animal House.

Systolic blood pressure was monitored in the conscious animal by the tail-cuff method using a programmed electro-sphygmomanometer PE-300 (Narco bio-instruments) coupled to a flat-bed record (CR-6505, J.J. instruments).

2.2ii Statistical Analysis

Results were analysed using Student's t-test for group and paired mean comparisons. Probability levels equal to or less than 0.05 were taken as indicating statistically

significant differences. All comparisons employed the two-tailed test.

2.3 Results

2.3i Table 2.1 compares the weight, systolic blood pressure, basal perfusion pressure and the pressor response to PNS and NA infusion in the six groups of rats.

The mean systolic pressures and the mean pressor responses to PNS and NA infusions were found to be significantly higher in the hypertensive group than those in their respective normotensive controls. The basal perfusion pressure was found to be essentially similar in all the groups. The males in general were found to be heavier than the age-matched females, though no significant difference was observed within the two male groups or the four female groups.

Besides weight, neither significant sex related differences nor strain (i.e. SH female versus NZH female and Wistar versus NZN) differences were observed between the groups.

Statistical tests were carried out to determine whether there was any correlation between the following parameters in each of the groups.

a) Weight and systolic blood pressure
b) Systolic blood pressure and basal perfusion pressure

Table 2.1

Systolic blood pressure and basal perfusion pressure, pressor responses to PNS and NA infusion in isolated mesenteric vascular beds from male and female SH (SHR_m and SHR_f, respectively), male and female Wistar (W_m and W_f, respectively) and female NZH and NZN rats

	Systolic blood pressure (mmHg)	Basal perfusion pressure (mmHg)	Pressor response to	
			PNS (30Hz, 1msec for 10 sec) (mmHg)	NA infusion (0.1 ml of 10^{-6}M) (mmHg)
SHR_m	195 ± 5 (25)	35 ± 3 (15)	15.0 ± 1.2 (19)	18.7 ± 0.8 (13)
W_m	98 ± 5 (20)	30 ± 2 (15)	6.9 ± 0.4 (20)	9.5 ± 0.5 (8)
SHR_f	185 ± 5 (25)	41 ± 4 (20)	14.1 ± 0.9 (20)	21 ± 0.17 (13)
W_f	94.2 ± 3 (25)	36 ± 5 (20)	7.2 ± 0.6 (20)	10.3 ± 0.5 (7)
NZH	181 ± 4 (25)	33 + 4 (20)	12.5 ± 1.4 (20)	17.4 ± 0.6 (13)
NZN	120 ± 5 (25)	36 ± 5 (20)	6.6 ± 1.2 (20)	8.6 ± 0.8 (10)

Values are given as mean ± s.e. of the mean.
Number in parenthesis = number of animals.

. indicates significant differences between SH., NZH rats and respective normotensive rats ($p<0.01$)

+ indicates signficant differences between SH, NZH rats and respective normotensive rats ($p<0.05$)

* indicates significant differences between SH, NZH rats and respective normotensive rats ($p<0.001$)

c) Basal perfusion pressure and pressor response to PNS and NA infusions

No correlation was found between any of the parameters.

2.3ii Effect of isoprenaline on the pressor response to PNS and NA infusion

Isoprenaline alone had no significant effect on the basal perfusion pressure of the isolated mesenteric preparation from any of the rat groups. In the preparations from the SH and NZH rats, isoprenaline caused a dose-dependant potentiation of the pressor response to PNS. At lower isoprenaline concentrations (10^{-9}M to $5x10^{-8}$M) a smaller potentiation of the pressor response to PNS was observed, whereas, at the higher isoprenaline concentrations (>$5x10^{-8}$M) inhibition of the vasoconstrictor response to PNS was observed in the normotensive control rats.
Figs 2.1 a,b,c.

A typical trace of the potentiating effect of isoprenaline on the PNS response in the hypertensive rat is shown in Fig 2.2

As can be seen in Figs 2.3 a,b,c, isoprenaline caused a dose dependant inhibition of the pressor response to NA infusion in both the hypertensive as well as the normotensive rats.

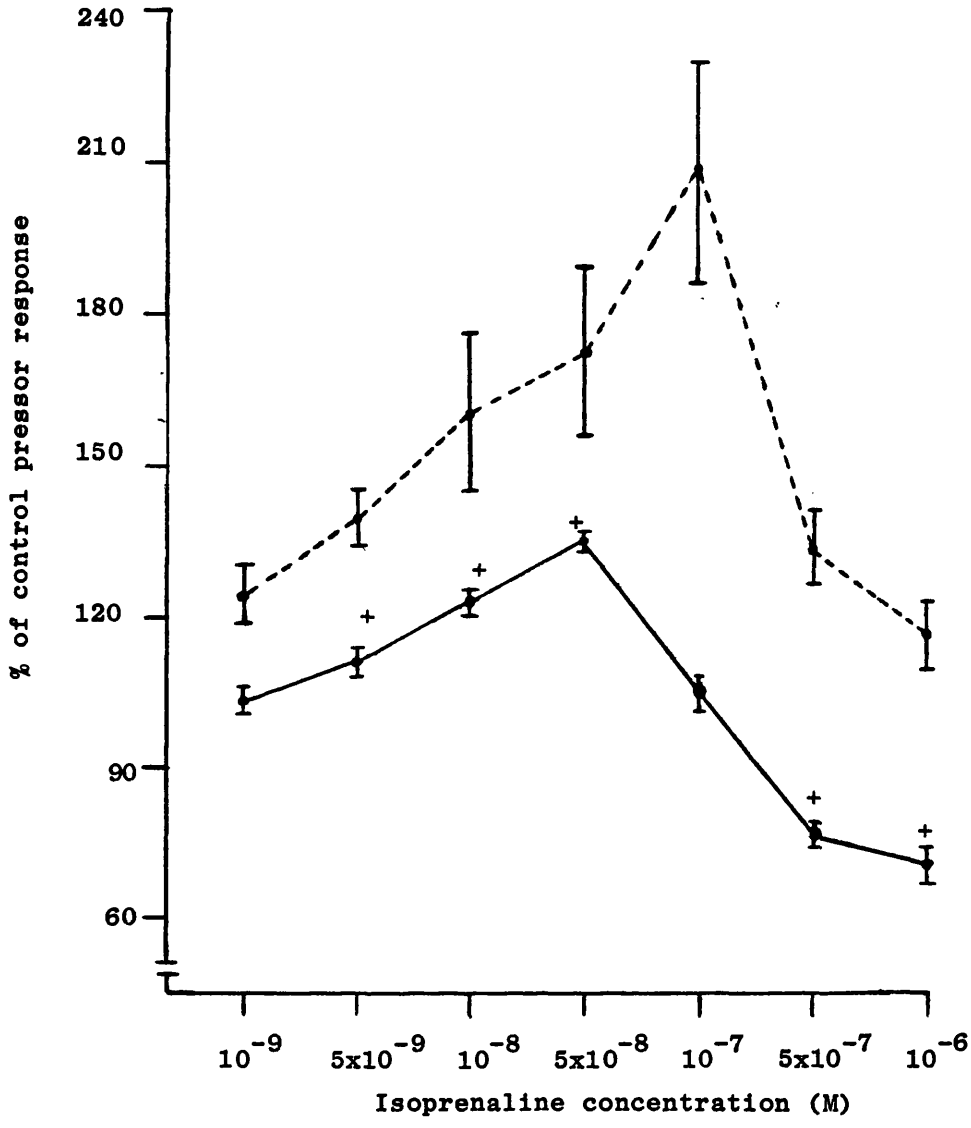


Fig 2.1a

Effect of isoprenaline on the pressor response to PNS in the isolated perfused mesenteric vasculature of male SH and (.---.) and male Wistar (•——•) rats.
Vertical lines indicate s.e. of mean. n = 4 animals for the SH group and n = 5 animals for the Wistar group
At all points, SH response significantly greater than Wistar response ($p<0.05$) and control pressor response ($p<0.01$)
+ $p<0.05$ compared to control pressor response

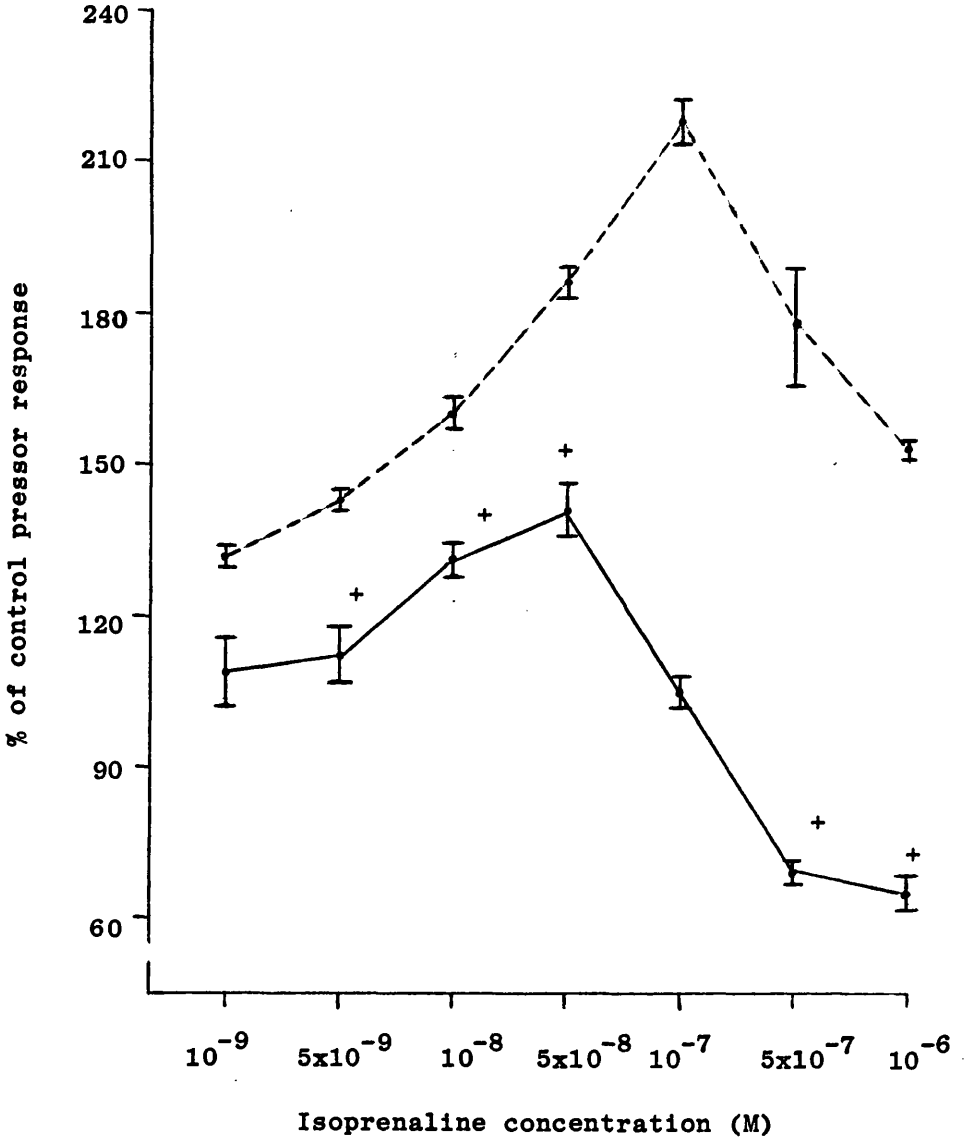


Fig 2.1b

Effect of isoprenaline on the pressor response to PNS in the isolated perfused mesenteric vasculature of female SH (.---.) and female Wistar (.____.) rats.
Vertical lines indicate s.e. of the mean.
n = 5 animals for the SH group and n = 4 animals for the Wistar group.
At all points, SH rat responses significantly greater than Wistar rat responses ($p<0.01$) and control responses ($p<0.01$).
+ $p<0.05$ compared to control pressor response.

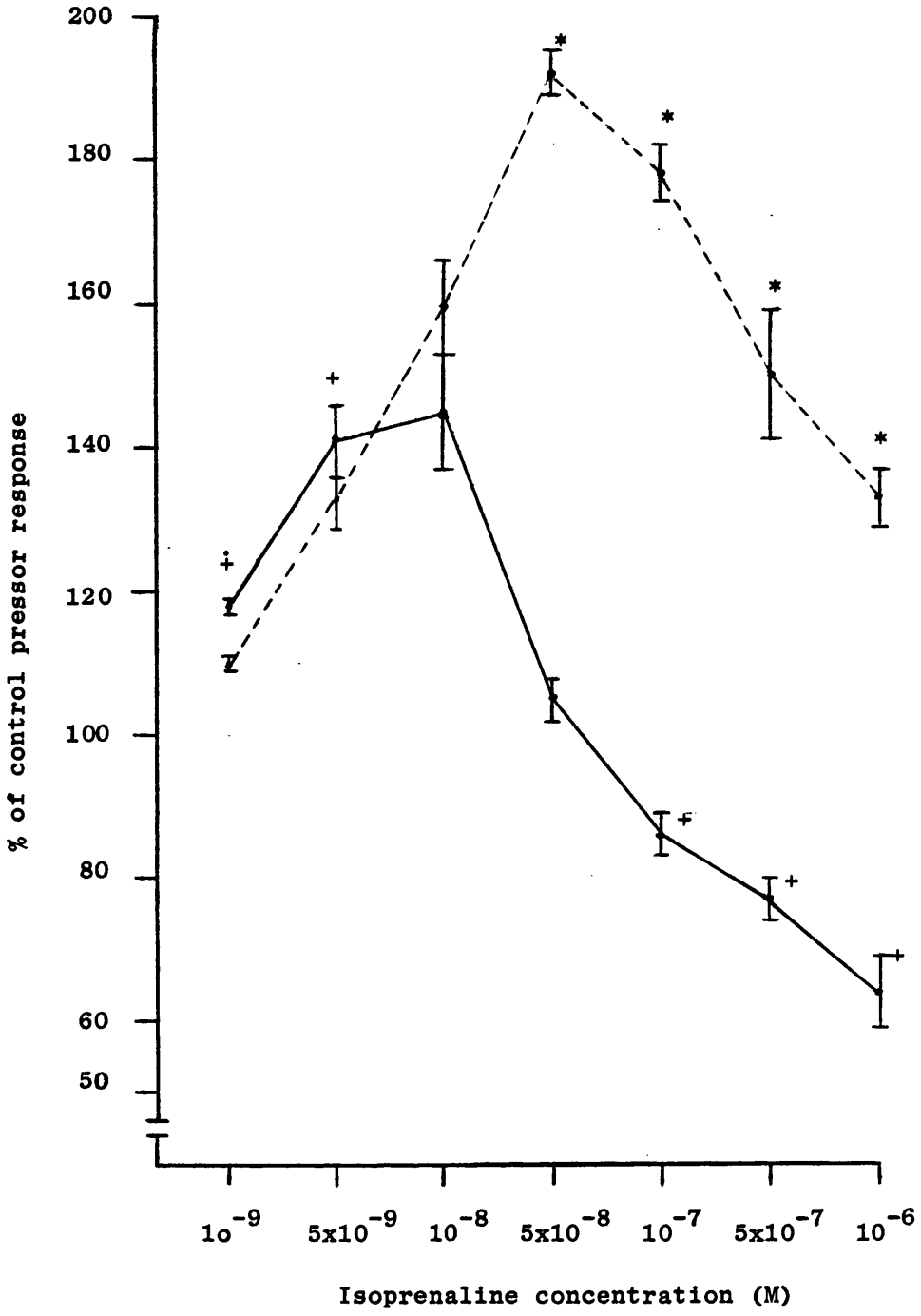


Fig 2.1c

Effect of isoprenaline on the pressor response to PNS in the isolated perfused mesenteric vasculature of female NZH (•---•) and female NZN (•——•) rats.
Vertical lines indicate s.e. of the mean.
n = 4 animals for each group
At all points, NZH rat responses significantly greater than control response ($p<0.05$)
+ $p<0.05$ compared to control pressor response
. $p<0.05$ compared to NZH rat
* $p<0.05$ compared to NZN rat

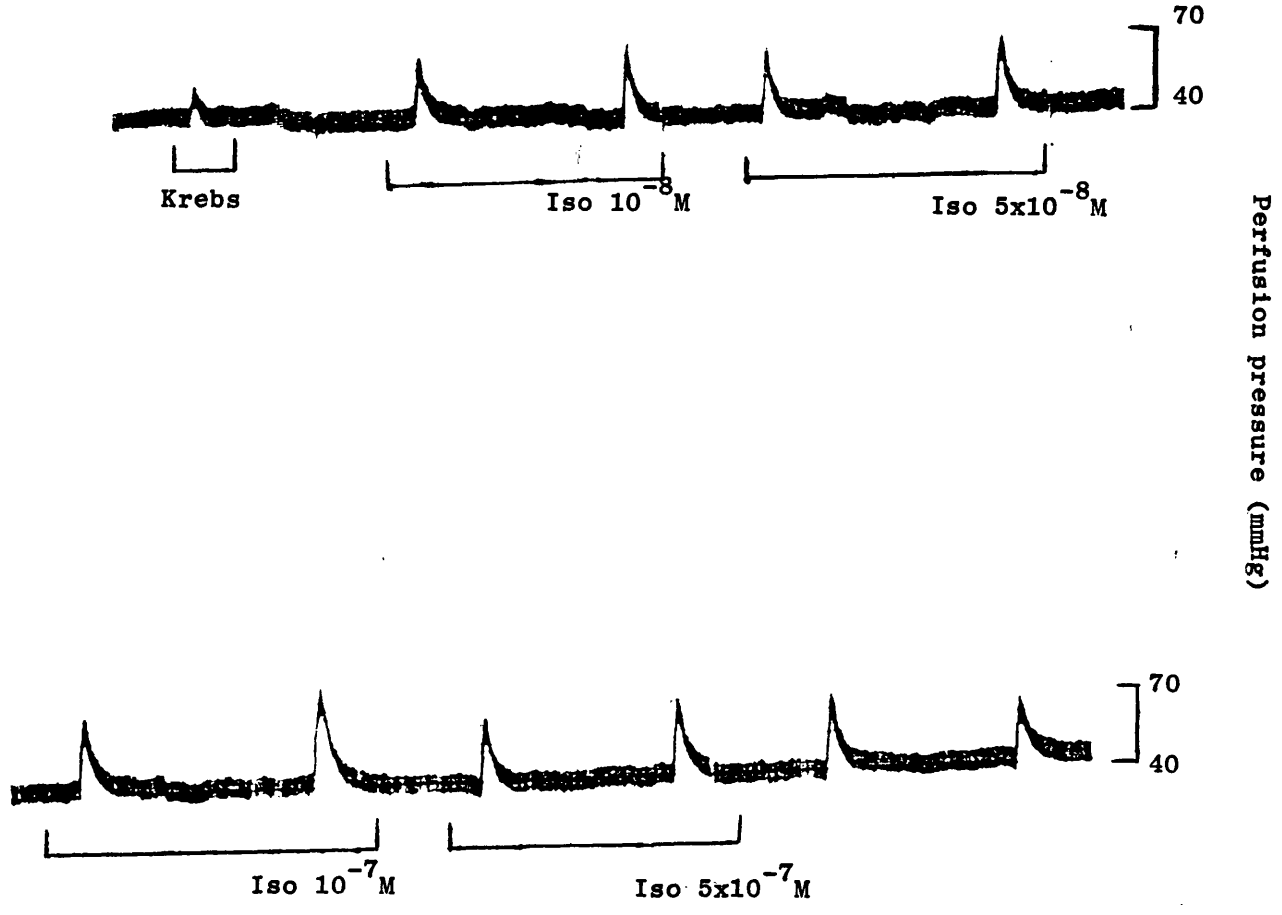


Fig 2.2. Typical trace of the facilitatory effect of Isoprenaline (Iso) on the pressor response to periarterial nerve stimulation in the isolated perfused mesenteric vasculature from female SH rat. Data dervied from the second response to periarterial nerve stimulation during perfusion of isoprenaline.

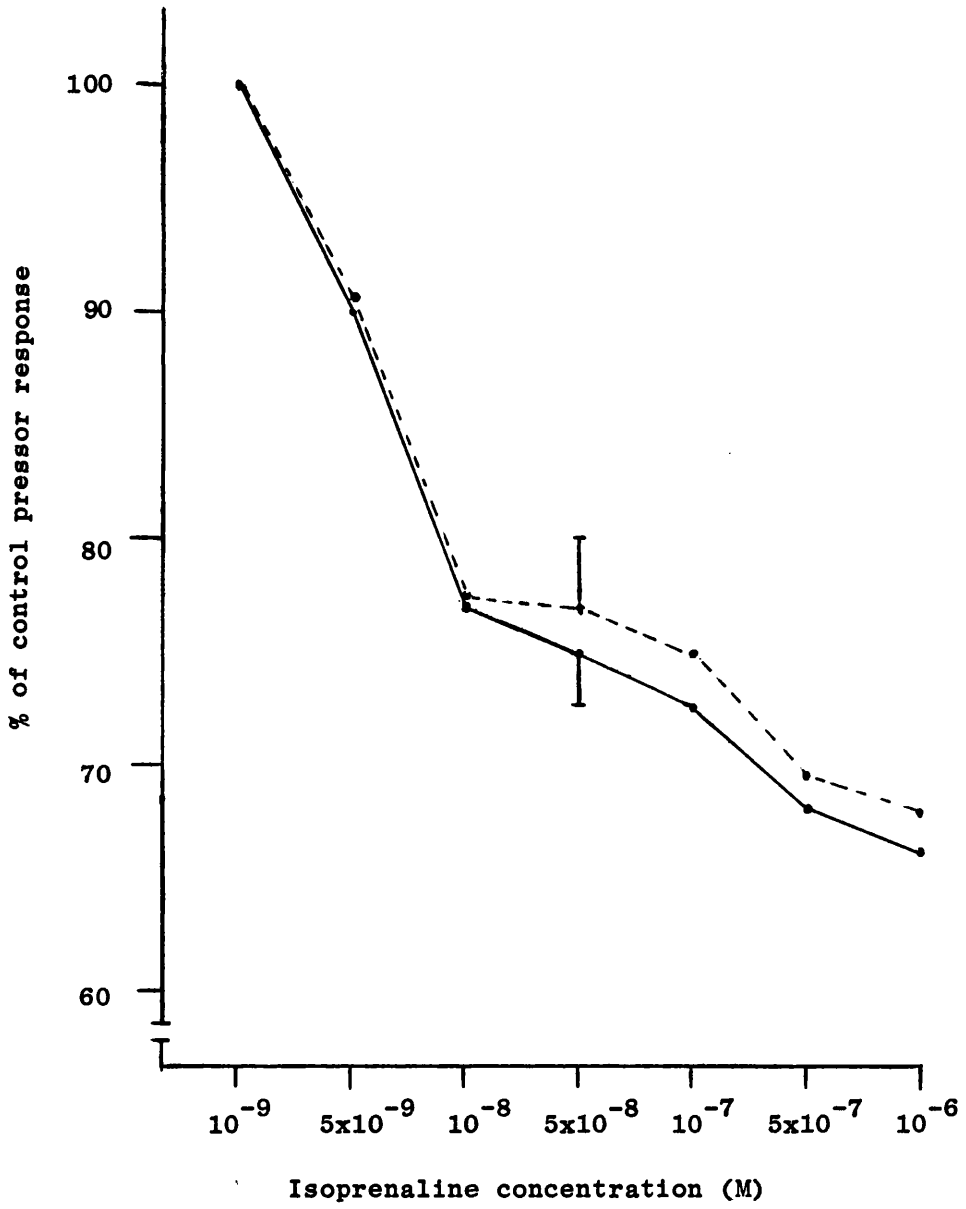


Fig 2.3a

Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused mesenteric vasculature of male SH (•---•) and male Wistar (•____•) rats.
Vertical lines indicate s.e. of mean (most omitted for clarity).
n = 4 animals for each group.

Responses from 5 x 10^{-9}M to 10^{-6}M isoprenaline significantly different from control pressor response ($p<0.05$).

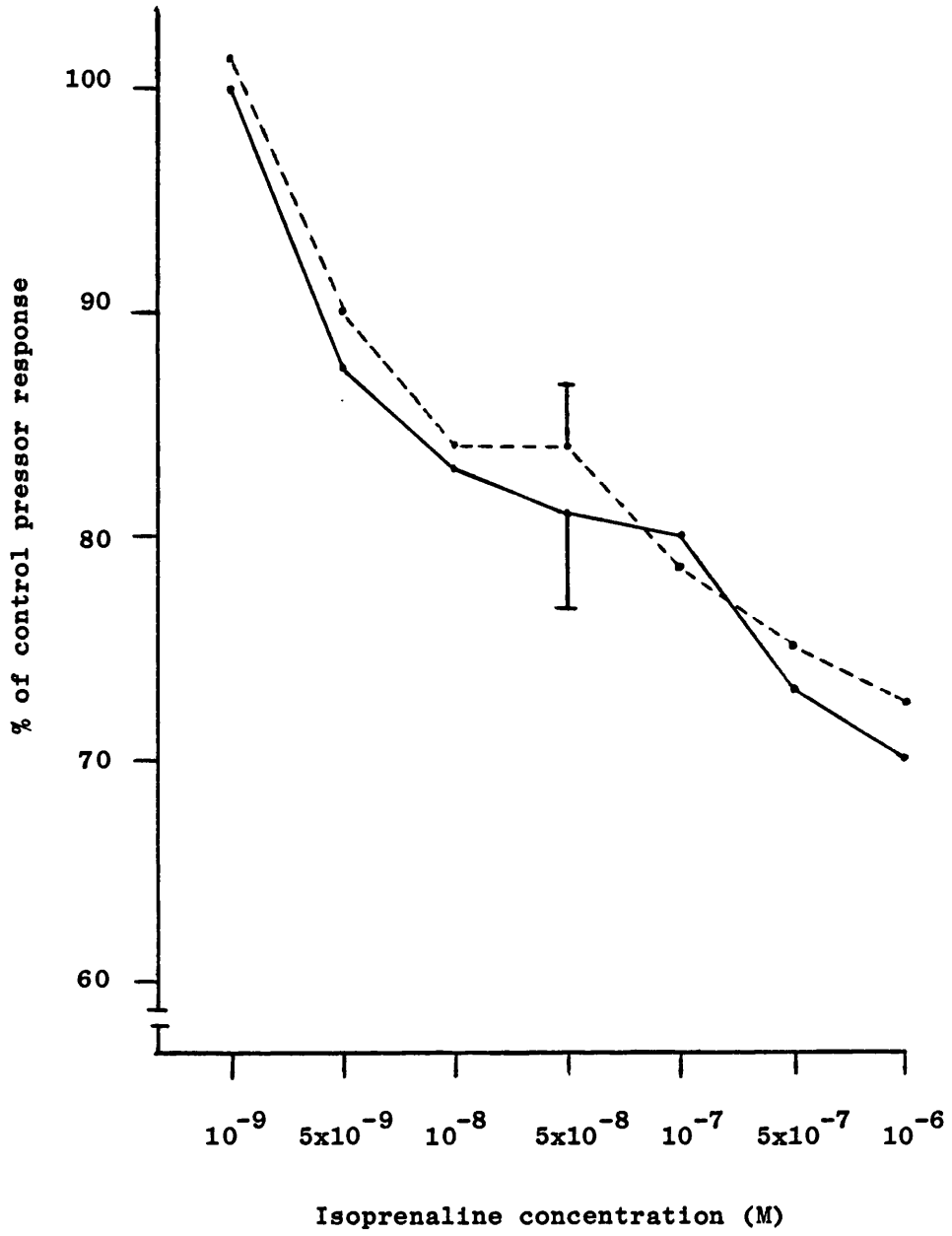


Fig 2.3b

Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused mesenteric vasculature of female SH (•---•) and female Wistar (•——•) rats.
Vertical lines indicate s.e. of the mean. Most omitted for clarity
n = 4 animals for the SH group and n = 3 animals for the Wistar group.
Responses from 5 x 10^{-9}M to 10^{-6}M isoprenaline significantly different from control pressor response ($p<0.05$)

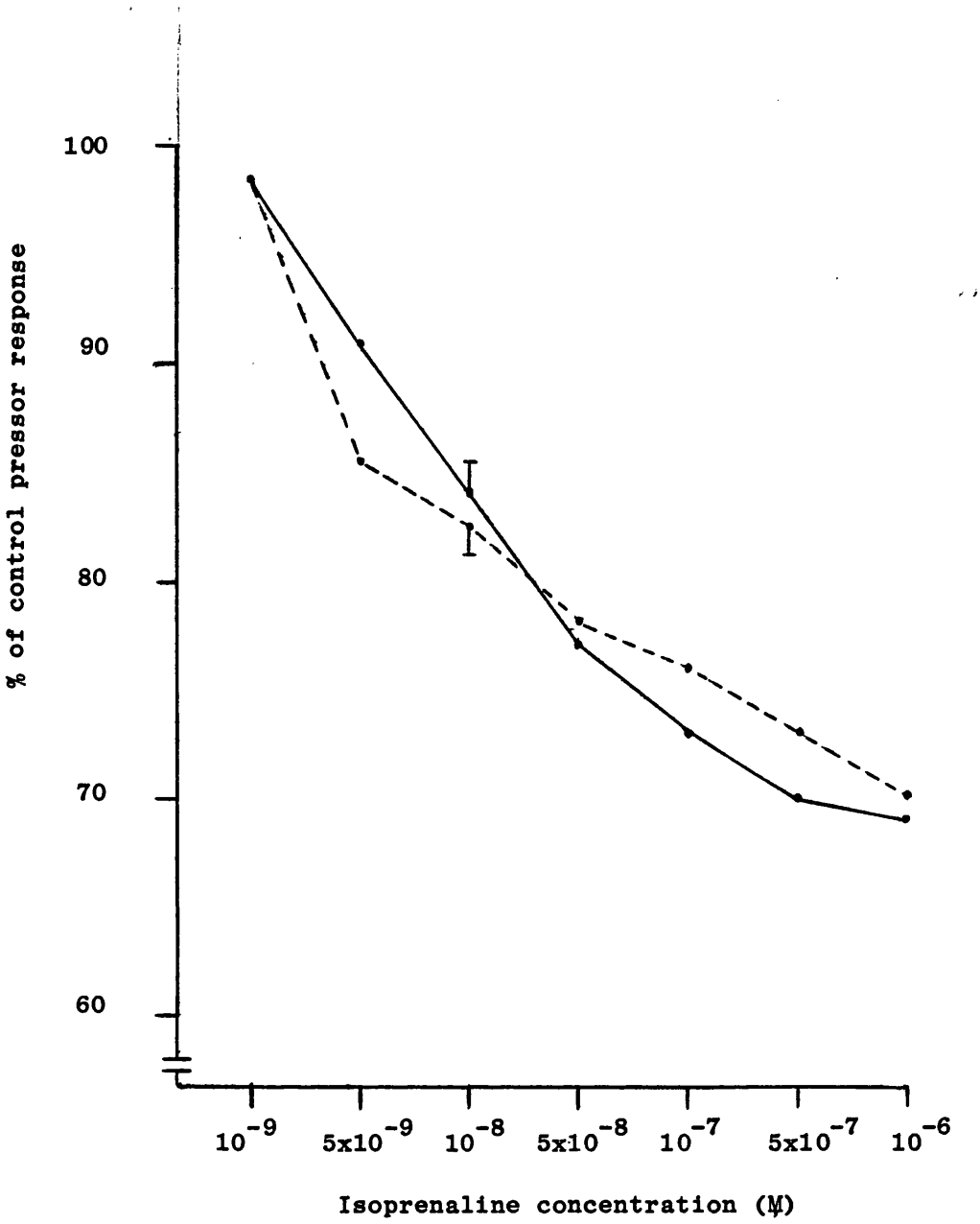


Fig 2.3c

Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused mesenteric vasculature of female NZH (.---.) and female NZN (._____.) rats.
Vertical lines indicate s.e. of the mean. Most omitted for clarity
n = 4 animals for each group.

Responses from 5 x 10^{-9}M to 10^{-6}M isoprenaline significantly different from control pressor response ($p<0.05$)

There was no significant difference in the degree of inhibition of the NA pressor response between the hypertensive and respective normotensive rats, however, the degree of the isoprenaline induced potentiation of the PNS pressor response was significantly higher, at all isoprenaline concentrations, in the hypertensive rats than in the respective normotensive rats.

In order to investigate the effects of β-adrenoreceptor antagonists, a standard concentration of $5x10^{-8}M$ isoprenaline was chosen, since this was the concentration that in control animals produced the greatest potentiation of the PNS pressor response.

2.3iii Effect of non selective beta adrenoreceptor antagonist on the isoprenaline induced effects on the pressor responses to PNS and NA infusions

Propranolol ($5x10^{-8}M$) was used as the non selective β-adrenoreceptor antagonist. At the concentration employed in this study, propranolol alone had no significant effect on the basal perfusion pressure when it was perfused through the preparation. Propranolol alone did not significantly affect the pressor response to PNS in any of the preparations but significantly inhibited the isoprenaline induced potentiation of the PNS pressor response in all the preparations Fig 2.4

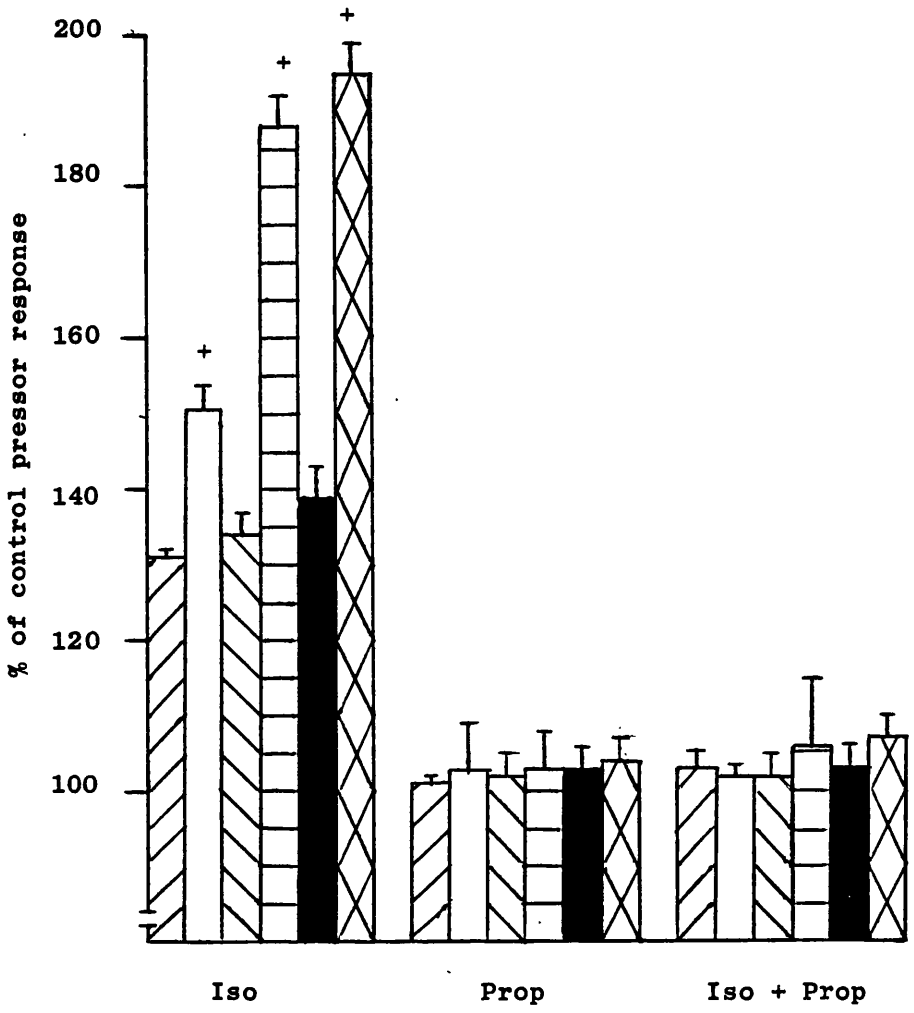


Fig 2.4

Effect of propranolol (Prop) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	n=8	Male Wistar rat	▨	n=4
Female SH rat	▤	n=8	Female Wistar rat	▧	n=3
Female NZH rat	▩	n=4	Female NZN rat	■	n=4

Vertical lines indicate s.e. of the mean.
n = number of animals for each group.
All groups in Iso section significantly different from control pressor response ($p<0.05$) and all other sections ($p<0.05$).
+ $p<0.05$ compared to respective normotensive animal.

Propranolol ($5x10^{-8}M$) by itself caused a moderate enhancement of the pressor response to NA infusion in all the rat groups. This enhancement, however, was not significant in the female NZN rats. The inhibitory effect of isoprenaline on the pressor response to NA infusion was significantly reversed by the propranolol in all the groups. Fig 2.5.

2.3iv Effect of selective β_1-adrenoreceptor antagonist on the isoprenaline induced effect on the pressor responses to PNS and NA infusion

Atenolol ($10^{-7}M$) was employed as the selective β_1-adrenoreceptor antagonist. At this concentration the atenolol had no significant effect on either the basal perfusion pressure or the pressor response to PNS in any of the preparations. Atenolol did not inhibit the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations. The results indicate that atenolol tended to potentiate further the isoprenaline induced potentiation of the pressor response to PNS in some groups, though this further potentiation was significant only in the male SH animals. Fig 2.6

Atenolol ($10^{-7}M$) alone tended to cause a moderate enhancement, about 11% over control, of the pressor response to NA infusion in the SH animals. Atenolol significantly reversed the inhibitory effect of

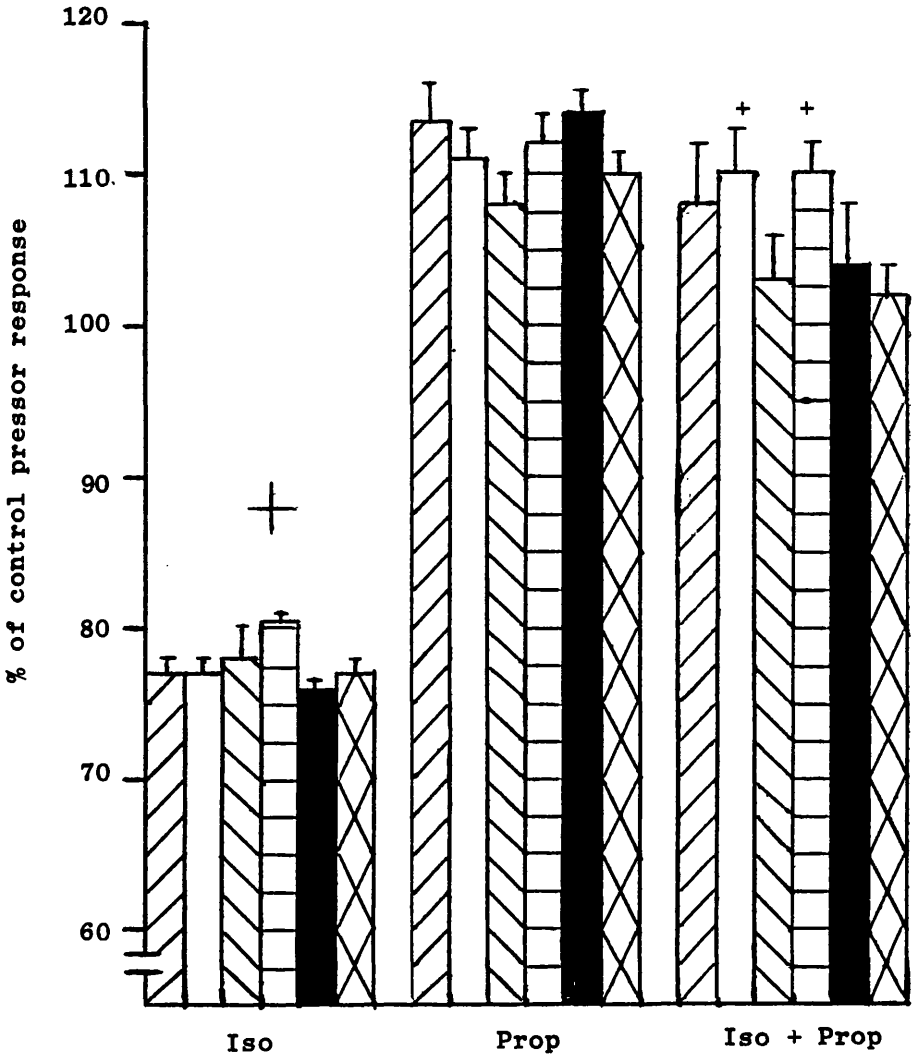


Fig 2.5

Effect of propranolol (Prop) on the isoprenaline (Iso)-induced inhibition of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	⧄
Female SH rat	⊟	Female Wistar rar	⧅
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group

+ $p < 0.05$, for groups in Iso section compared to control pressor response and groups in other sections.
+ $p < 0.05$ compared to control pressor response
All groups in Prop section significantly different compared to control pressor response ($p < 0.05$)

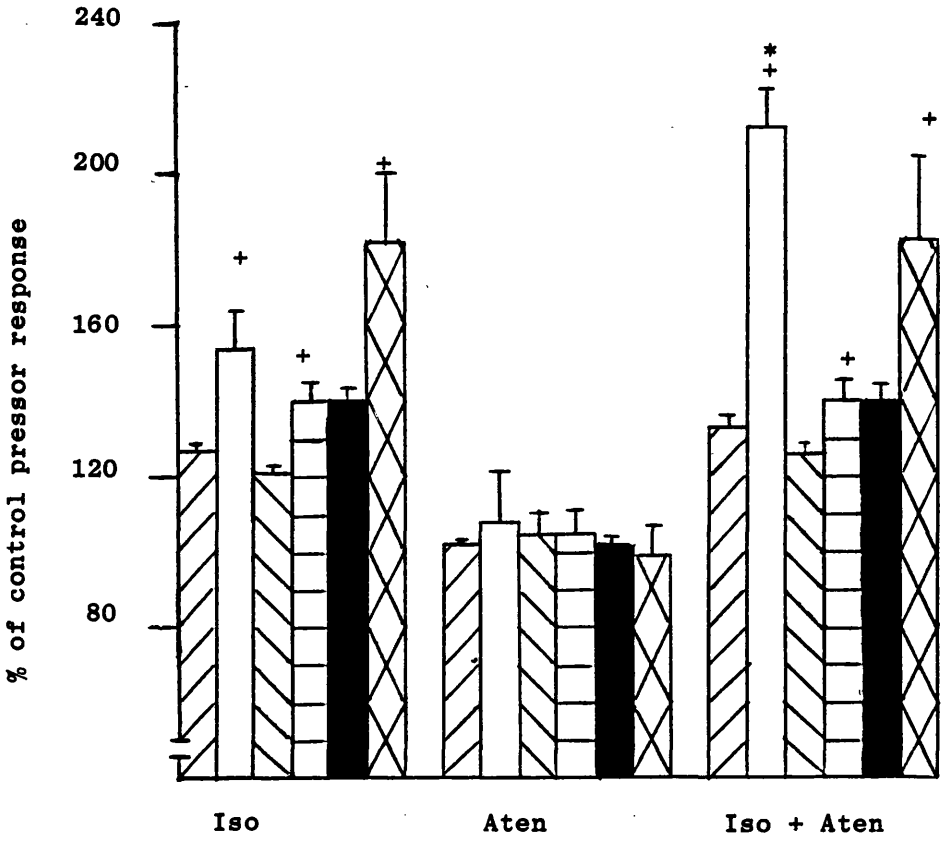


Fig 2.6

Effect of atenolol (Aten) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▭	Female Wistar rat	▧
Female NZH rat	☒	Female NZN rat	■

Vertical lines indicate s.e of the mean.
n = 4 animals for each group.
All groups in isoprenaline section and isoprenaline + atenolol section, $p<0.05$, compared to control pressor response and atenolol section.
+ $p<0.05$ compared to respective normotensive rats
* $p<0.05$ compared to isoprenaline alone.

isoprenaline on the pressor response to NA infusion in the preparations from all the groups of rats. Fig 2.7.

2.3v Effect of selective β_2-adrenoreceptor antagonist on the isoprenaline induced effects on the pressor responses to PNS and NA infusions

ICI 118,551 ($5x10^{-7}M$) was used as the selective β_2-adrenoreceptor antagonist. At this concentration the antagonist had no significant effect on either the basal perfusion pressure or the pressor response to PNS in any of the groups of rats. However, the ICI 118,551 completely inhibited the isoprenaline induced facilitation of the PNS pressor response in the preparations from both the hypertensive and control animals. Fig 2.8.

ICI 118,551 significantly inhibited the isoprenaline induced inhibition of the pressor response to NA infusion in all the preparations. Fig 2.9.

2.3vi Effect of angiotensin II on the pressor response to PNS and NA infusion

Angiotensin II (10 ng/ml) tended to produce a transient and generally non-significant increase in basal perfusion pressure of about 15% in some of the preparations. Angiotensin II (AII) significantly potentiated the pressor response to both PNS and NA infusion in all the groups. The potentiating effect of angiotensin II on the PNS

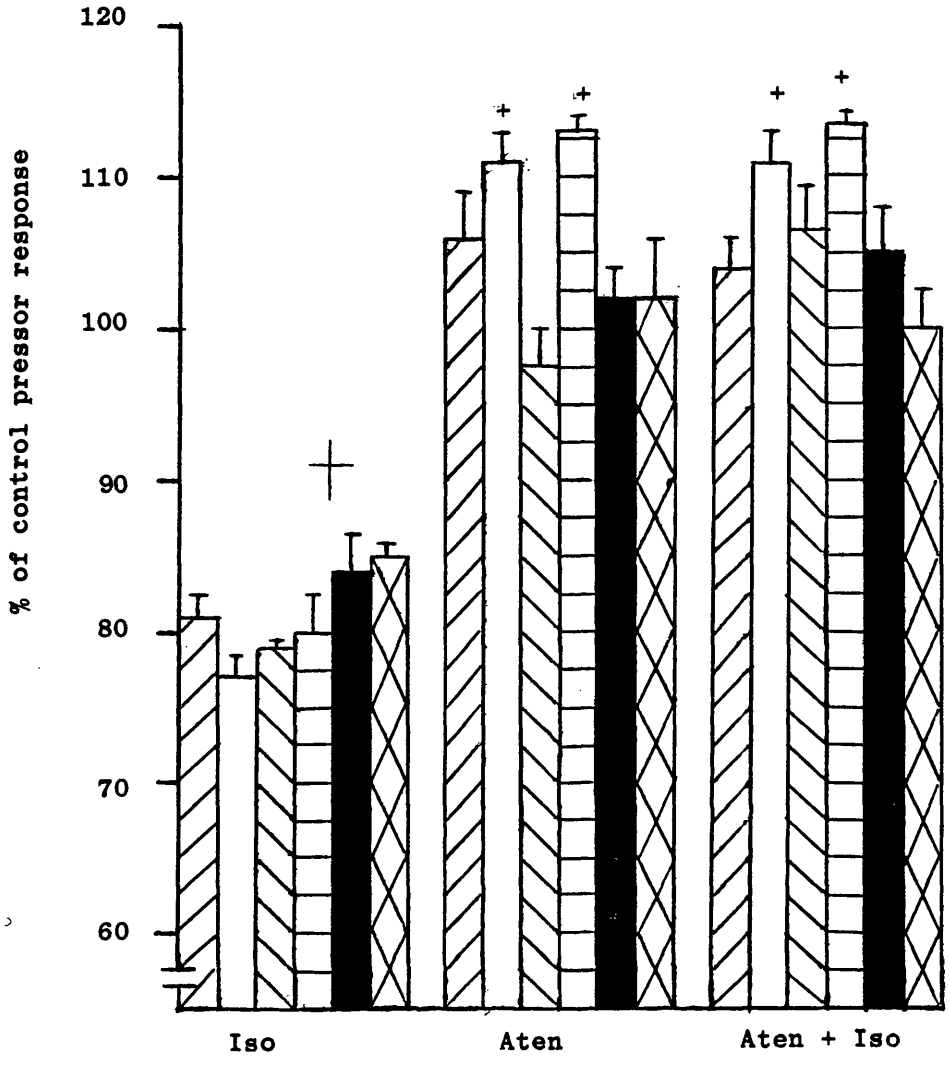


Fig 2.7.

Effect of atenolol (Aten) on the isoprenaline (Iso)-induced inhibition of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature from SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▤	Female Wistar rat	▧
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p<0.05$, for groups in Iso section compared to control pressor response and same group in other sections.
+ $p<0.05$ compared to control pressor response.

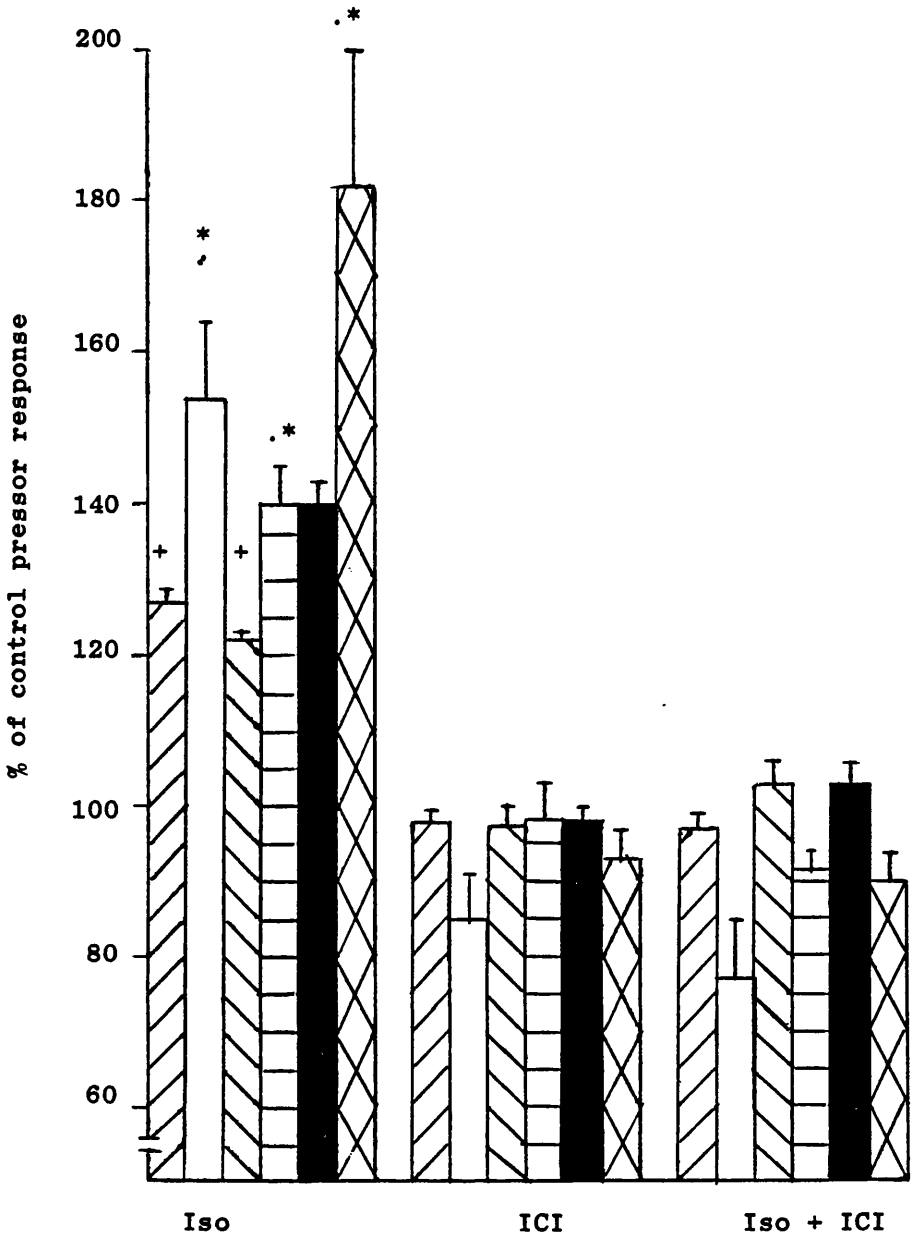


Fig 2.8

Effect of ICI 118,551 (ICI) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▭	Female Wistar rat	▧
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 4 animals for each group.
* $p<0.05$ compared to normotensive rats
. $p<0.01$ compared to control pressor response and same group in other sections
+ $p<0.05$ compared to control pressor response and same group in other sections.

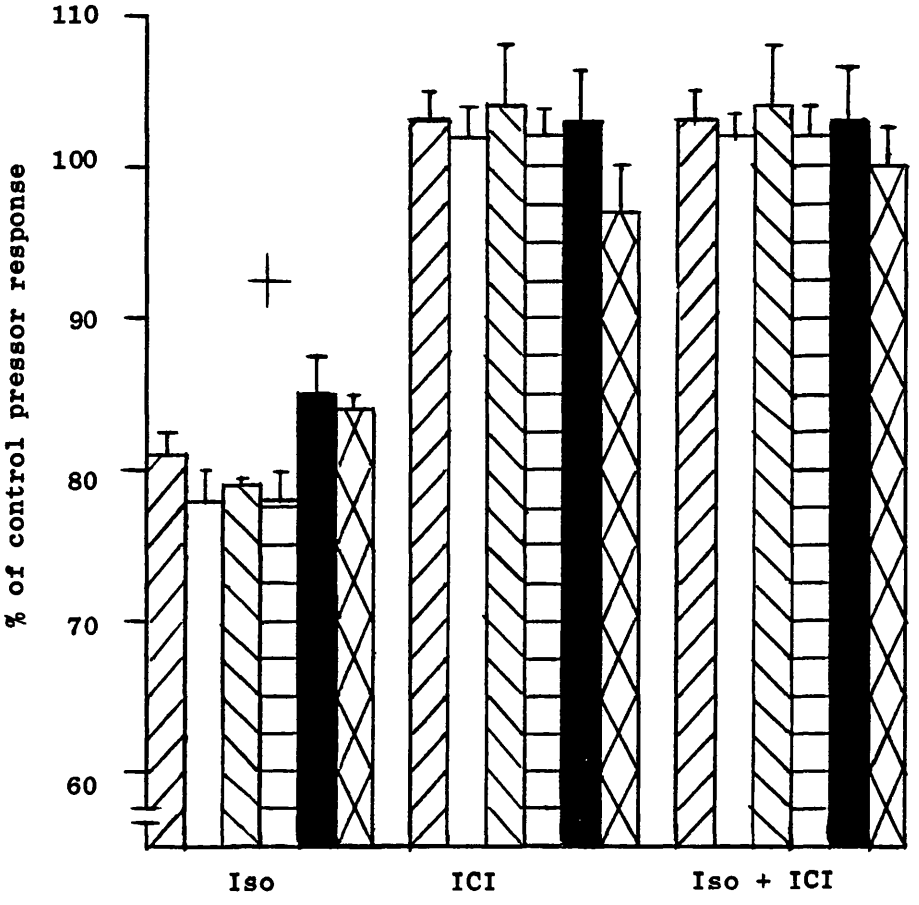


Fig 2.9

Effect of ICI 118,551 (ICI) on the isoprenaline (Iso)-induced inhibition of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature from SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▭	Female Wistar rat	▧
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p<0.05$ compared to control pressor response and to groups in other sections.

response was found to be significantly greater in the SH and NZH preparations than that in the respective control preparations. Fig 2.10.

The degree of potentiation caused by the angiotensin II on the pressor response to NA infusion was found to be essentially similar in all the groups. Fig 2.11.

In the male SH and all the female preparations the degree of potentiation of the pressor response to PNS was significantly greater than that of the NA infusion response ($p<0.05$).

The specific angiotensin II receptor antagonist, [Sar^1-Ile^8] angiotensin II (Sar, 200 ng/ml), which by itself did not significantly effect the basal perfusion pressure and the pressor responses to PNS and NA infusion, completely prevented the potentiation of the PNS and NA infusion responses caused by angiotensin II (10 ng/ml). Fig 2.10. and 2.11.

Figs 2.12 and 2.13 shows the effect of cocaine (5 µg/ml), which had no effect on the basal perfusion pressure, on the pressor response to PNS and NA infusion respectively. Cocaine significantly potentiated the pressor response to PNS and produced a moderate potentiation of the pressor response to NA infusion in the preparations from both the hypertensive and normotensive animals. The degree of potentiation of the PNS pressor response in the SH and NZH

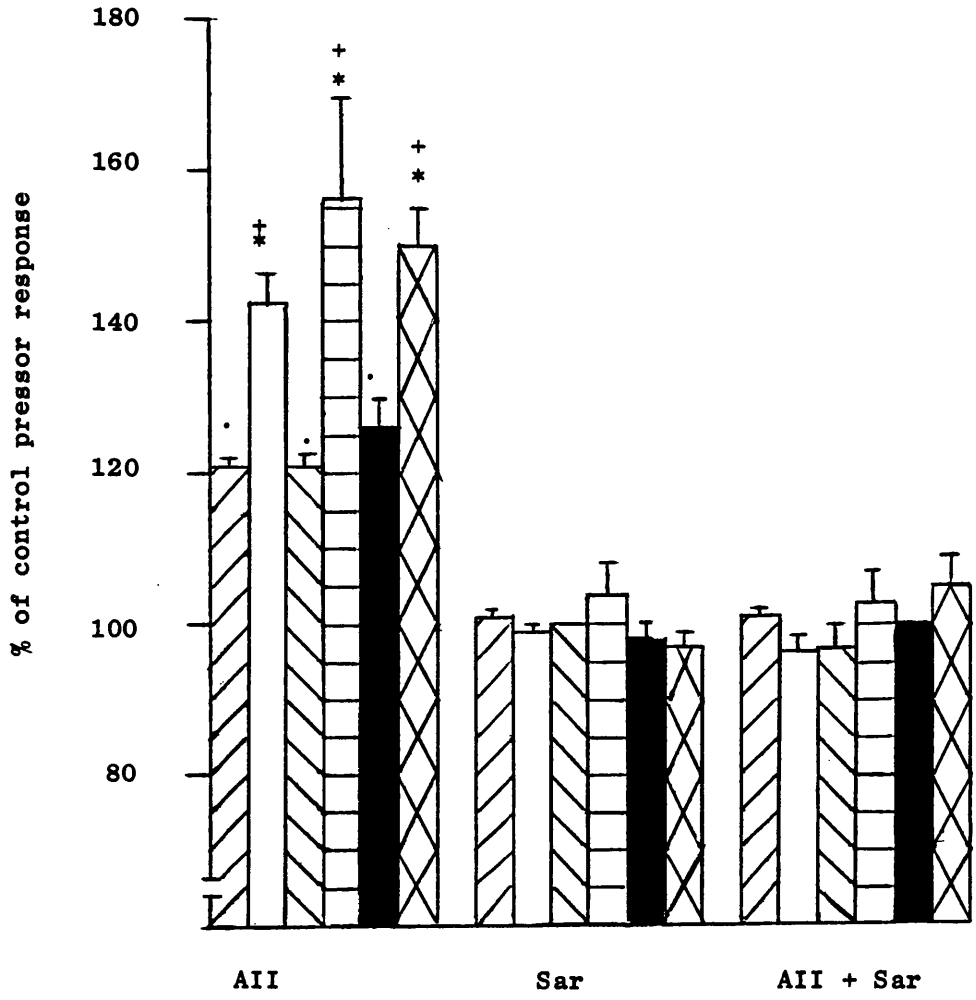


Fig 2.10

Effect of angiotensin II (AII) and $[Sar^1\text{-}Ile^8]$ angiotensin II (Sar) on the pressor response to PNS in the isolated mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	n=4	Male Wistar rat	▨	n=4
Female SH rat	▤	n=3	Female Wistar rat	▧	n=4
Female NZH rat	⊠	n=5	Female NZN rat	■	n=3

Vertical lines indicate s.e of mean.
n = number of animals for each group.
. $p<0.05$, * $p<0.01$ compared to control pressor response and other sections.
+ $p<0.05$ compared to respective normotensive rats.

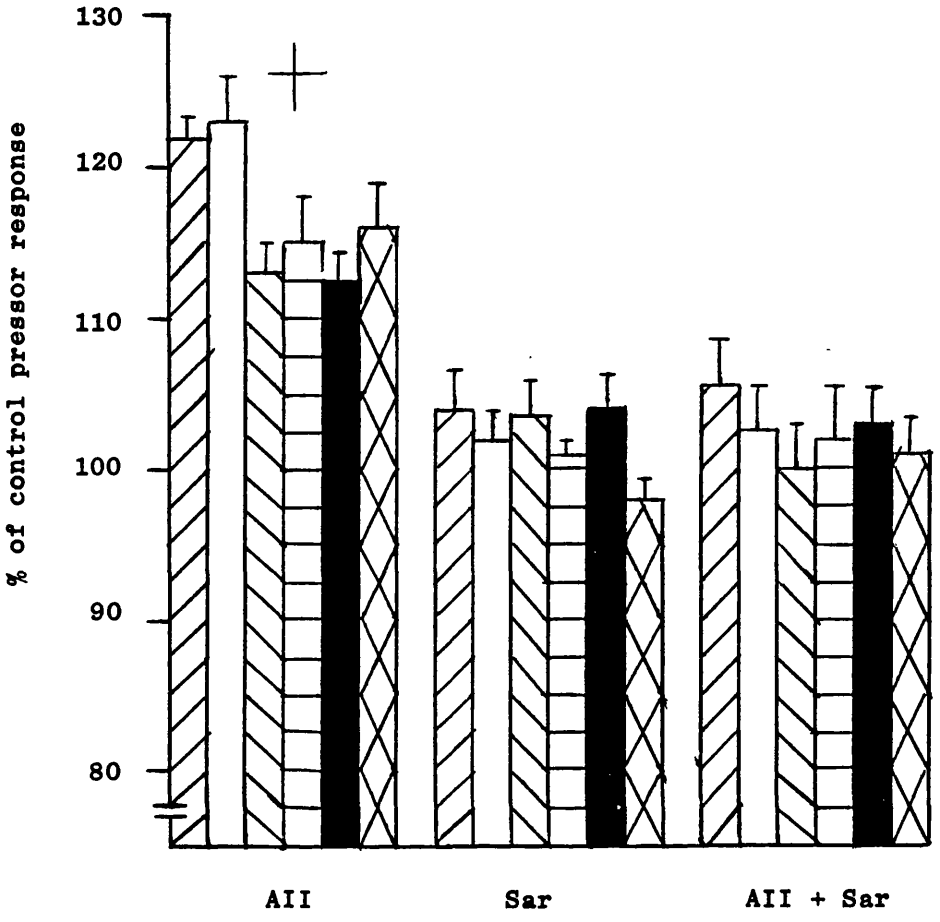


Fig 2.11

Effect of [Sar^1-Ile^8] angiotensin II (Sar) and angiotensin II (AII) on the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature from SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▤	Female Wistar rat	▧
Female NZH rat	▩	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p < 0.05$ for groups in AII section compared to control pressor response and other groups.

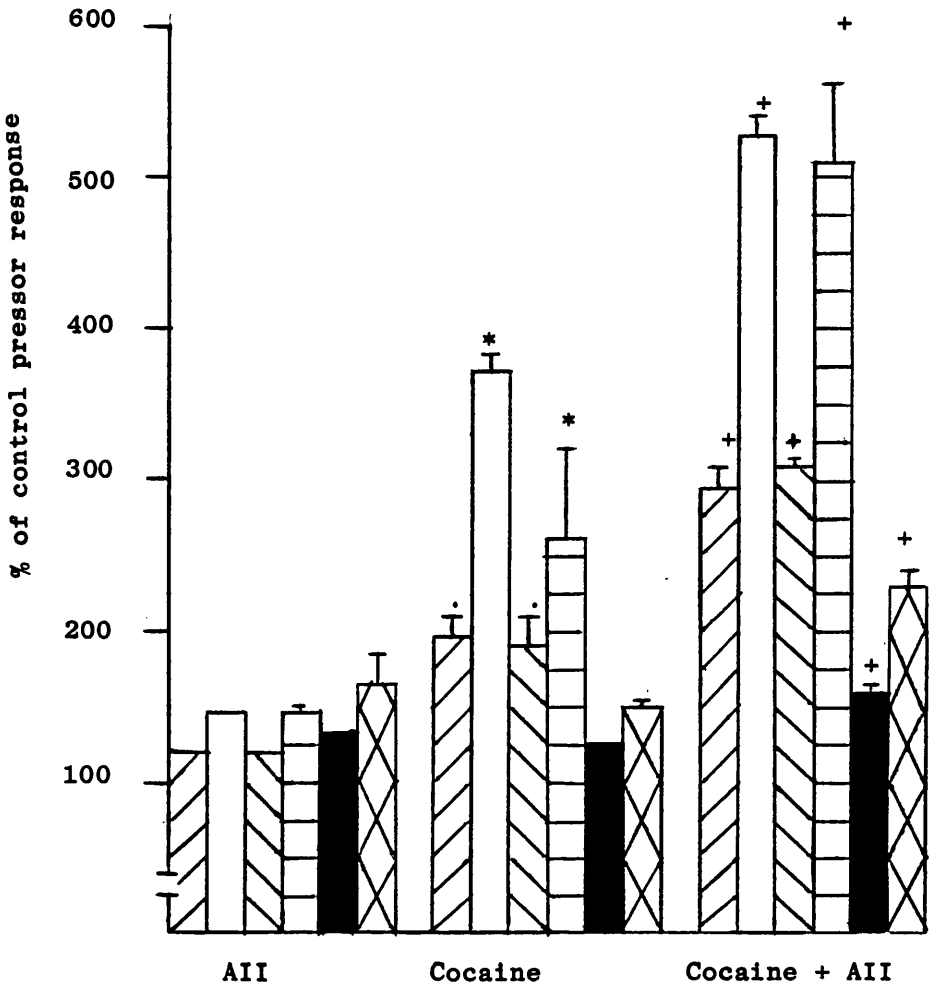


Fig 2.12

Effect of cocaine on the angiotensin II (AII)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rat.

Male SH rat	□	Male Wistar rat	⧄
Female SH rat	⊟	Female Wistar rat	⧅
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e of the mean.
n = 4 animals for each group.
All sections, hypertensive rats compared to respective normotensive rats, $p<0.01$.
+ $p<0.05$ compared to cocaine section
. $p<0.05$, * $p<0.01$ compared to AII section

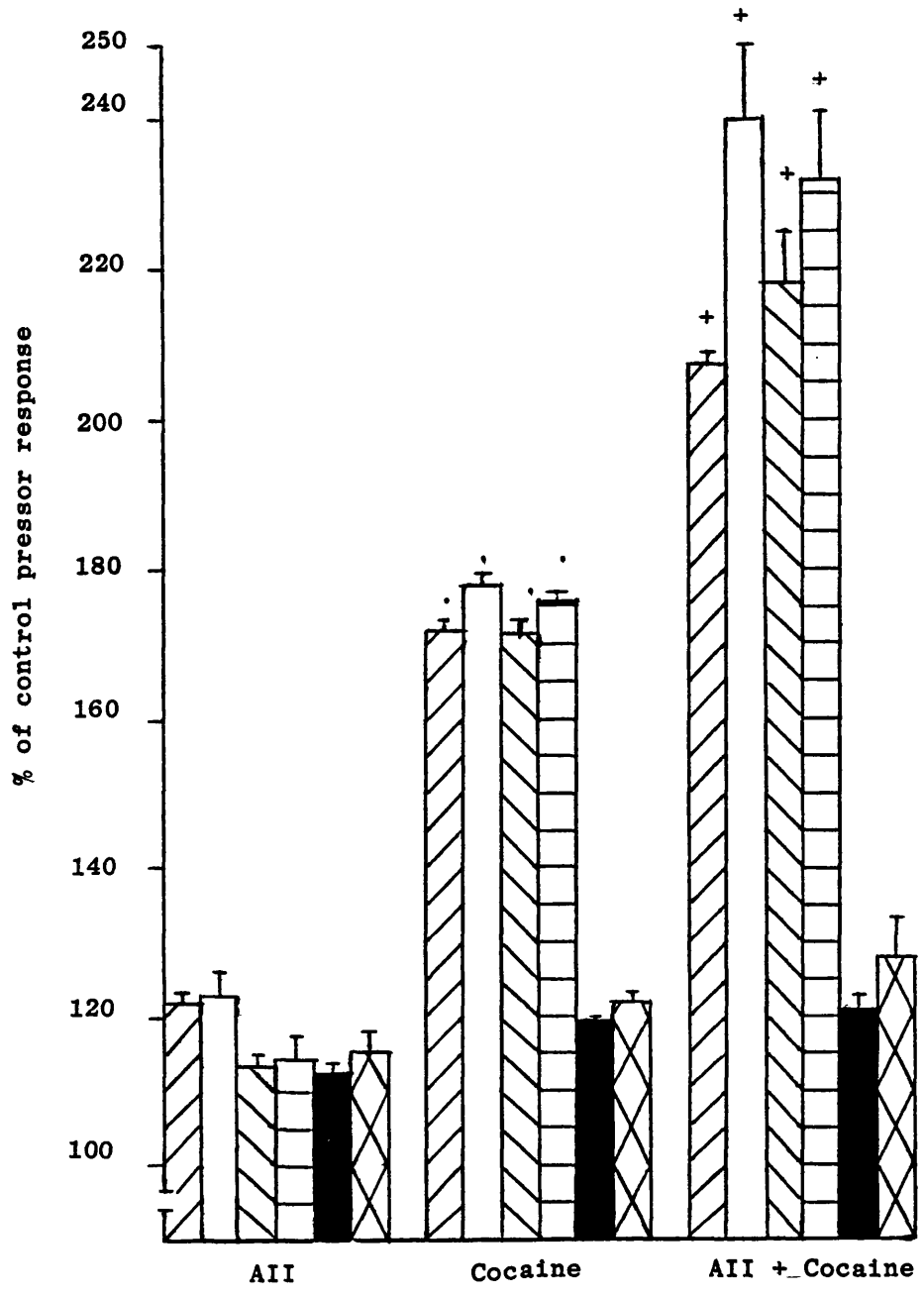


Fig 2.13

Effect of cocaine on the angiotensin II (AII)-induced potentiation of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▭	Female Wistar rar	▧
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p<0.05$ compared to cocaine section
. $p<0.05$ compared to AII section

preparations was significantly greater than that in the respective control preparations, whereas no significant differences between the preparations from hypertensive and normotensive animals were observed in the pressor responses to NA infusion.

When angiotensin II (10 ng/ml) was administered together with cocaine (5 µg/ml),the potentiation of the pressor response to both PNS and NA infusion was potentiated further and appeared to be additive. The facilitatory effect of angiotensin II on the PNS response was greater in the SH and NZH than in the Wistars and NZN preparations in the presence of cocaine, as well as in its absence.

Thus far these results indicate that, in preparations from male and female animals, the vasoconstrictor responses to PNS are potentiated by β-adrenoreceptor stimulation and by activation of angiotensin II receptors and that this potentiation is greater in the SH and NZH animals than in the Wistars and NZN animals. The next series of experiments explore the relationship between the β-adrenoreceptor and the angiotensin II receptor.

2.3vii Effect of captopril on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Captopril ($5x10^{-6}M$) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS

and NA infusion. However, captopril effectively inhibited the isoprenaline induced potentiation of the pressor response to PNS in all the preparations, whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion in any of the preparations. Figs 2.14 and 2.15.

2.3viii Effect of [Sar^1-Ile^8] angiotensin II on the isoprenaline induced affects on the pressor responses to PNS and NA infusions

Sar (200 ng/ml) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion. However, Sar effectively inhibited the isoprenaline induced potentiation of the pressor response to PNS in all preparations, whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion in any of the preparations. Figs 2.16 and 2.17.

2.3ix Effect of ICI 118,551 on the angiotensin II (100 ng/ml) potentiation of the pressor response to PNS and NA infusion

As shown earlier (Fig 2.8 and 2.9), ICI 118,551 ($5x10^{-7}M$) alone had no significant effect on the basal perfusion pressure or pressor responses to PNS and NA infusions. As shown in Fig 2.18 and 2.19 ICI 118,551 had no significant effect on the angiotensin II potentiation of

the pressor responses to PNS and NA infusion in any of the preparations from any of the groups.

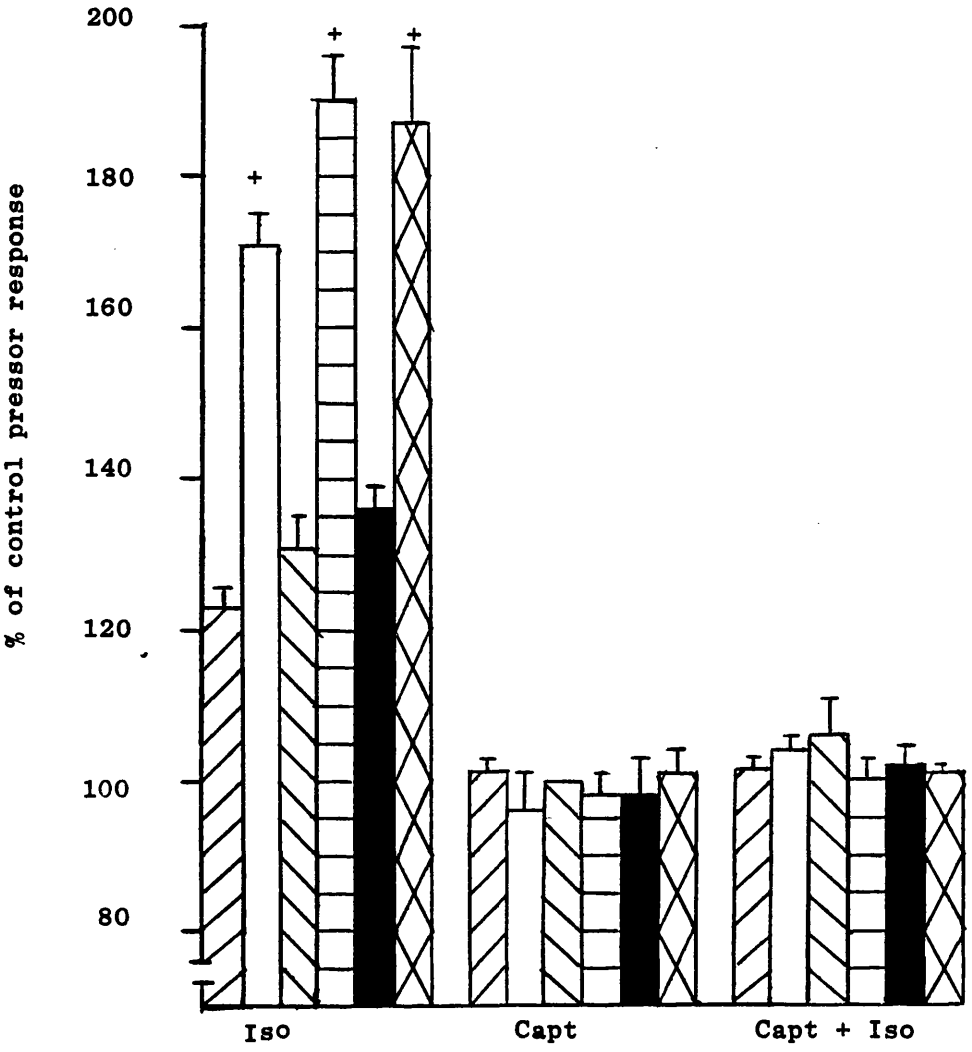


Fig 2.14

Effect of captopril (Capt) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature from SH,Wistar, NZH and NZN rats.

Male SH rats	□	Male Wistar rats	▨
Female SH rats	▤	Female Wistar rats	▧
Female NZH rats	⊠	Female NZN rats	■

Vertical lines indicate s.e. of the mean.
n = 4 animals for each group.
All groups in Iso section significantly greater than groups in other sections, $p<0.05$. + $p<0.05$ compared to normotensive rats.

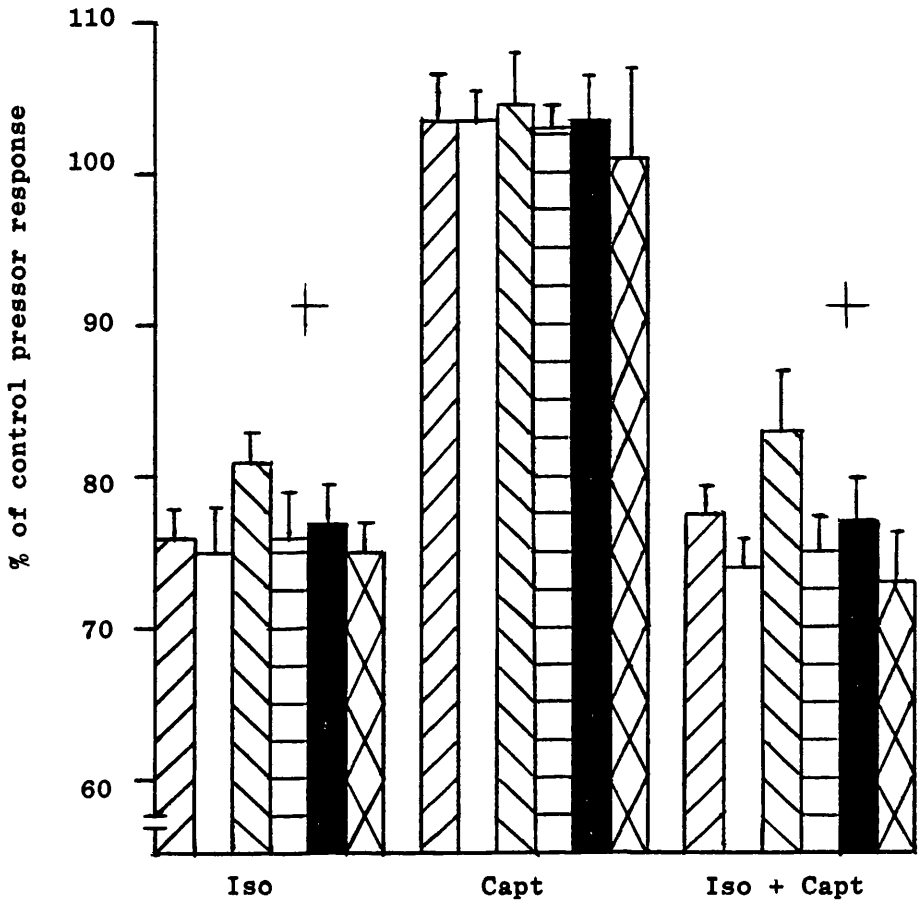


Fig 2.15

Effect of captopril (Capt) on the isoprenaline (Iso)-induced inhibition of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rats	□	Male Wistar rats	▨
Female SH rats	▭	Female Wistar rats	▧
Female NZH rats	☒	Female NZN rats	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p<0.05$, for groups in Iso and Iso + Capt section compared to control pressor response and to Capt section

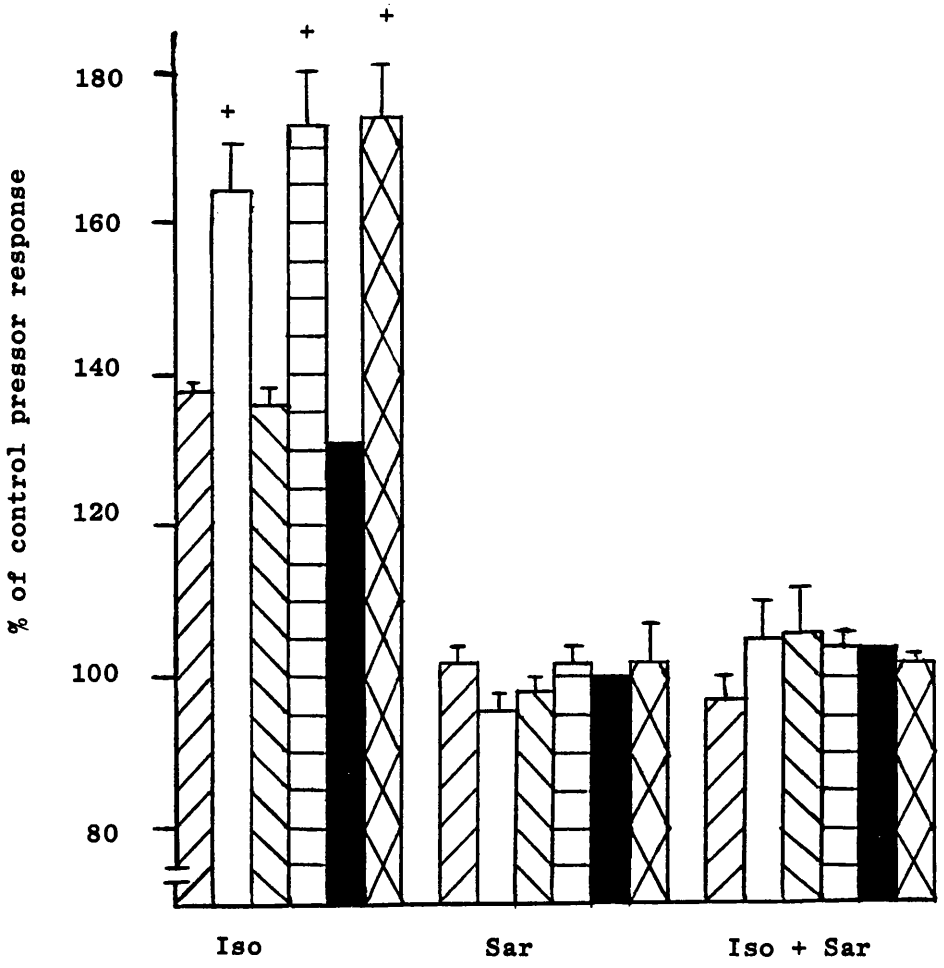


Fig 2.16

Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature from SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▤	Female Wistar rat	▧
Female NZH rat	▩	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
All groups in Iso section significantly different to control pressor response and to groups in other sections ($p<0.05$).
+ $p<0.05$ compared to normotensive rats.

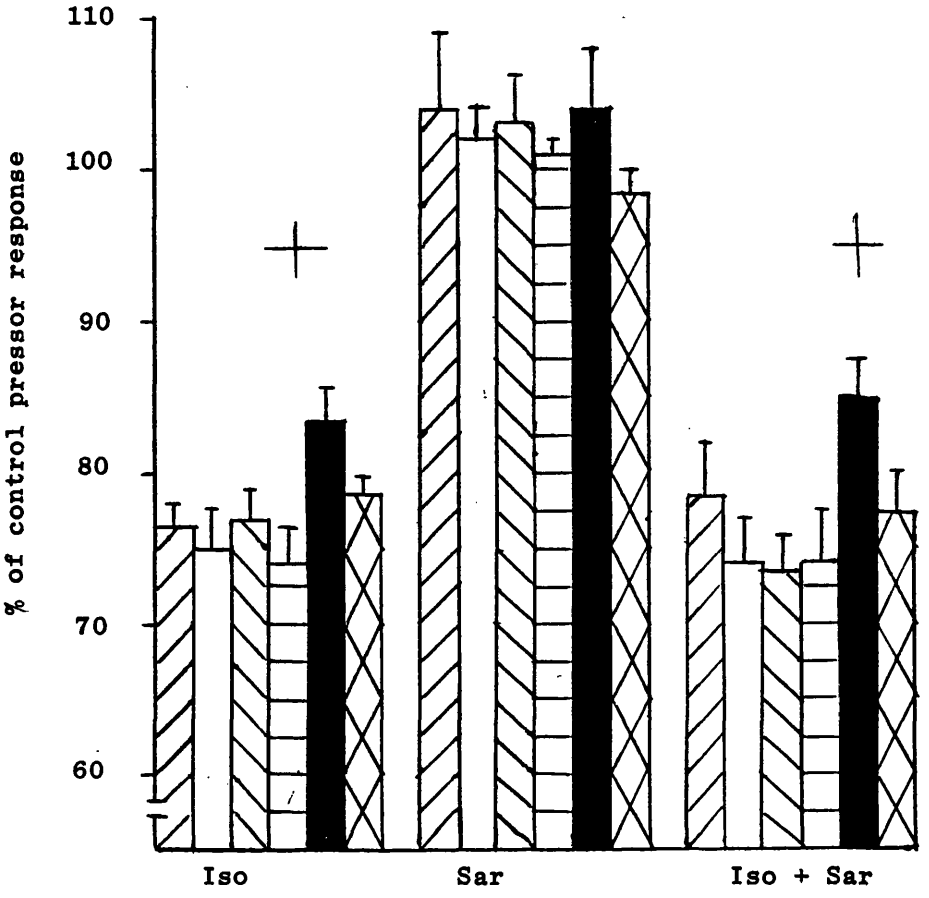


Fig 2.17

Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced inhibition of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▤	Female Wistar rat	▧
Female NZH rat	▩	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
+ $p < 0.05$, for groups in Iso and Iso + Sar section compared to Sar section and control pressor response.

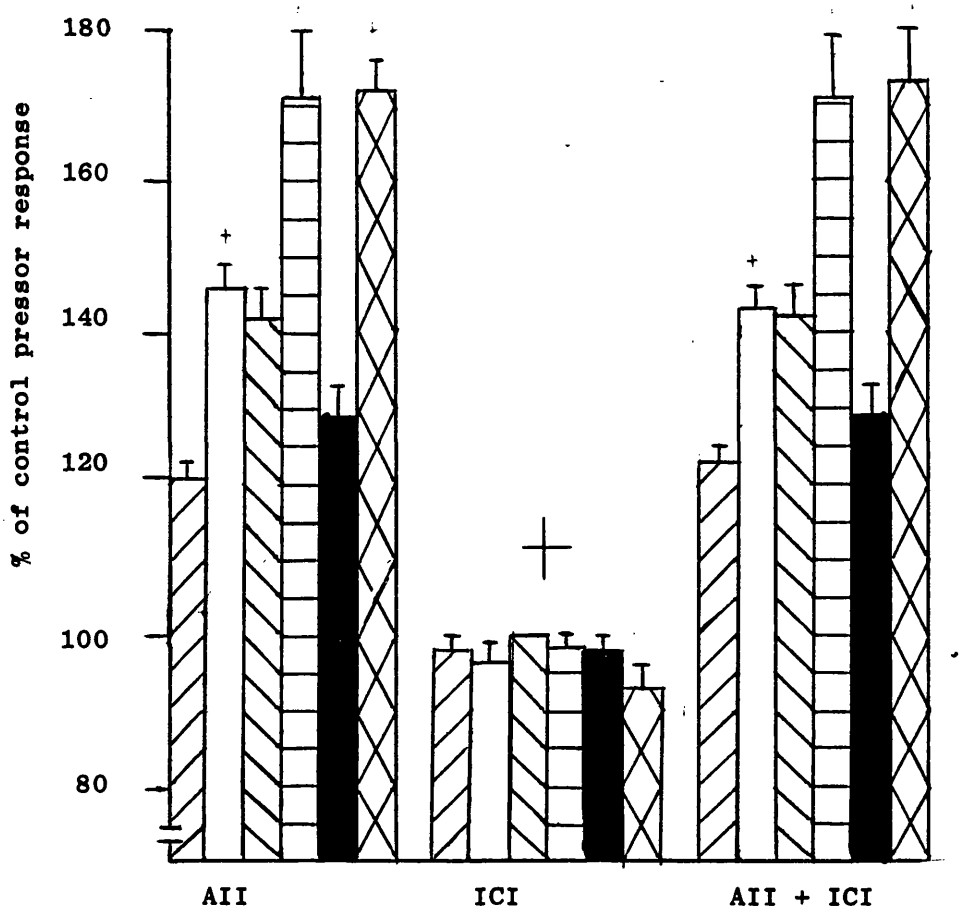


Fig 2.18

Effect of ICI 118,551 (ICI) on the angiotensin II (AII)- induced potentiation of the pressor response to PNS in the isolated mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat □ Male Wistar rat ▨

Female SH rat ▭ Female Wistar rat ▧

Female NZH rat ⊠ Female NZN rat ■

Vertical lines indicate s.e. of the mean.
n = 3 animals for each group.
┼ $p<0.05$, groups in this section compared to other sections.
\+ $p<0.05$ compared to respective normotensive rat.

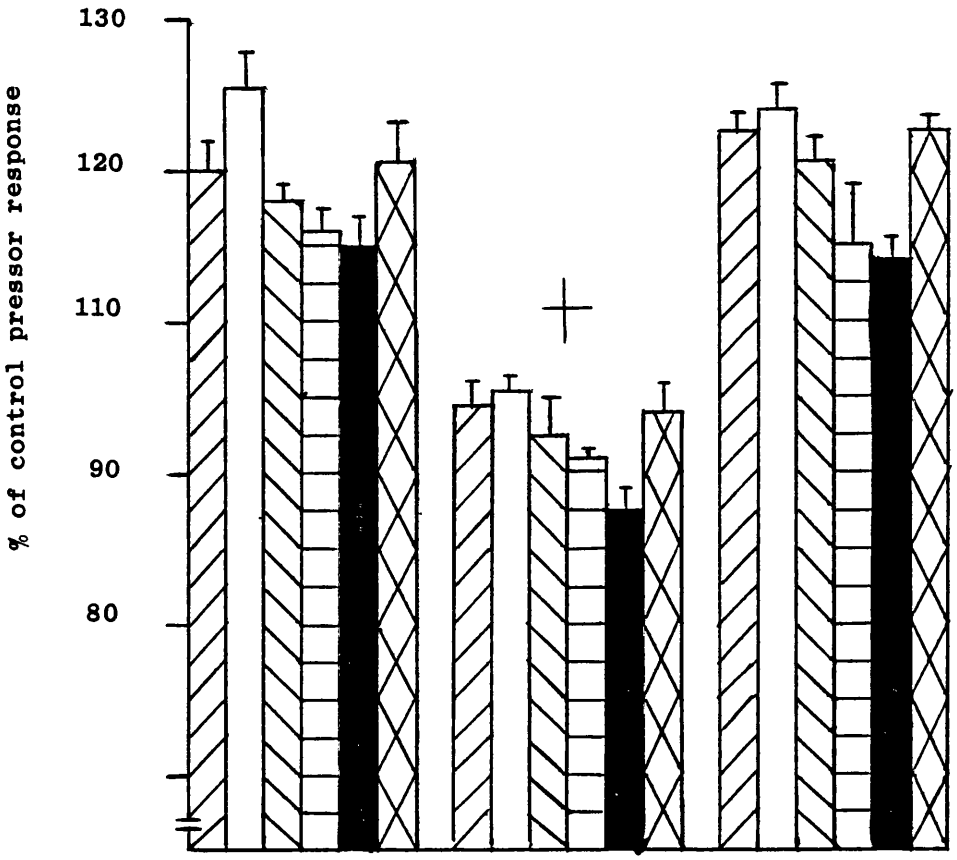


Fig 2.19

Effect of ICI 118,551 (ICI) on the angiotensin II (AII)-induced potentiation of the pressor response to noradrenaline infusion in the isolated perfused mesenteric vasculature of SH, Wistar, NZH and NZN rats.

Male SH rat	□	Male Wistar rat	▨
Female SH rat	▤	Female Wistar rat	▧
Female NZH rat	⊠	Female NZN rat	■

Vertical lines indicate s.e. of the mean.
n = animals for each group.
+ $p < 0.05$, for groups in ICI section compared to other sections.

2.4 Discussion

The results described in this chapter suggest that physiological mechanisms facilitating sympathetic neurotransmission in the mesenteric vascular bed are enhanced in the hypertensive rats compared to the normotensive controls. This facilitation seems to be qualitatively and quantitatively similar in male and female SH rats and in female NZH rats. That the facilitatory mechanisms are located preynaptically can be inferred from the comparison of the effects of isoprenaline and angiotensin II on the pressor responses to PNS and to infused exogenous NA. It can be further concluded that the augmentation of presynaptic activity by isoprenaline is mediated via β_2-adrenoreceptors. The results also imply that the sequence of events is first the activation of a local renin-angiotensin system following β_2-adrenoreceptor stimulation, rather than vice versa, and suggesting that a local renin-angiotensin system, is involved in mediating β_2-adrenoreceptor mediated events via presynaptic angiotensin II receptors in the mesenteric vascular bed.

Both the local facilitatory systems are measurably more sensitive in the SH and NZH preparations than in the Wistar and NZN preparations and are equally marked in both male and female animals.

The preparations from NZH rats seem to be less sensitive to the effects of cocaine than preparations from SH rats. This could imply that in the vascular preparations from NZH rats there may be a less cocaine-sensitive mechanism for the uptake of NA and that this could account for the increased vasoconstriction found in the NZH rat compared to that in the NZN rat.

The next chapter is devoted to a series of experiments conducted in a different tissue to see whether the findings reported in this chapter are generally applicable or are true only for the mesenteric vascular bed.

CHAPTER 3

ISOLATED PERFUSED KIDNEY

3.1 Preliminary Work

The isolated kidney was set up as described below and perfused with oxygenated Krebs. However, I found that the preparation did not respond consistently to PNS for more than 30 minutes. Tyrode solution was substituted in place of Krebs and it was found that the kidney preparation responded consistently to PNS for over 60 minutes. The isolated kidney seemed more sensitive to PNS when Tyrode was perfused through it than when Krebs was used.

Ideal parameters for PNS in order to give consistent and repeatable pressor responses were found to be 3Hz, 50V, 1 msec pulse width for 5 seconds. Next, a concentration of NA necessary to give an increase in perfusion pressure of the preparation roughly similar to that caused by PNS (3Hz, 50V, 1msec, 5sec) was determined; this concentration was found to be 0.1ml of a 10^{-7}M solution of noradrenaline bitartarate.

3.2 Method

3.2i Isolated perfused rat kidney

The rats were anaesthetised with an intra-peritoneal injection of pentobarbitone sodium (0.1ml of 6% W/V solution per 100g body weight). An abdominal incision was made in the midline, extended ventrally and laterally, and the intestines were reflected to the left. The right kidney and major abdominal vessels were exposed by clearing away the fat and perivascular tissue by blunt dissection. The aorta was gently separated from the inferior vena cava around the area of the bifurcation of the right renal artery from the aorta. The right renal artery was also gently freed from the right renal vein. Care was taken not to damage the renal nerves, and the renal vasculature was kept moist with oxygenated Tyrode solution.

The adrenal branch of the right renal artery was tied and loose ligatures were placed around the following blood vessels:-

(1) the superior mesenteric artery, near the aorta

(2) the aorta, above the bifurcation of the superior mesenteric artery.

(3) the right renal artery, near the aorta.

In addition, cotton thread was passed around the inferior vena cava, near the bifurcation of the right renal artery, and the right renal vein. This was done so that the vena cava and the right renal vein could be gently pulled away from the aorta and the right renal artery respectively, prior to cannulation of the right renal artery.

Next, the ligature round the mesenteric artery was tied off followed by the aortic ligature. As soon as the aortic ligature was tied a small incision was made in the aorta at the point of the right renal artery's bifurcation from the aorta, and a portex 17 gauge (3FG) polyethylene cannula, outer diameter 0.75mm, filled to the tip with heparin (1000 units/ml) was inserted into the right renal artery and the ligature was tied, thus securing the cannula in the renal artery. A total of 200 units of heparin (1000 units/ml) was injected into the renal artery and then oxygenated Tyrode solution was allowed to perfuse through the right renal artery and kidney at the rate of 10ml/min.

The right kidney was then removed from the rat by cutting off at the aorta, the renal vein, the ureters, the adrenal gland and the connective tissue around the kidney, and was placed in a 5ml water-jacketed organ bath maintained at 37°C. The isolated kidney was perfused with oxygenated Tyrode at a constant rate of 10ml/min. by means of a Watson Marlow peristaltic pump. The time that elapsed

between ligating the aorta and the beginning of the Tyrode perfusion of the kidney was never more than 40 seconds.

The Tyrode solution was of the following composition (mM):-

Sodium Chloride,	137;	Potassium Chloride,	2.7;
Calcium Chloride,	1.8;	Magnesium Chloride,	1.0;
Sodium bicarbonate,	11.9;	Sodium hydrogen phosphate,	0.4;
Glucose,	5.5;	Ascorbic acid,	0.1

The perfusing solution was aerated with a mixture of 95% oxygen and 5% carbon dioxide before passing through a warming coil maintained at 38°C. Changes in perfusion pressure were measured at a point close to the cannula by means of a pressure transducer (Bell and Howell, type 4-422-0001) and recorded on a Devices (M2) recorder.

After allowing time for the basal perfusion pressure to stabilise, usually 15 minutes, the isolated perfused kidney was subjected to either periarterial nerve stimulation (PNS) or to a bolus of NA. PNS was delivered at 5 minute intervals via bipolar platinum electrodes placed around the renal artery. Supramaximal rectangular pulses, 1msec, 50V, were applied for 5 seconds at 3Hz by means of a Grass, model S44, stimulator. The neural basis of the pressor response mediated by stimulation of the arterial adrenergic nerve was confirmed by abolition of the response after perfusion with

guanethidine (0.01mM, n=3 animals for each group). Noradrenaline (0.1ml of 10^{-7}M) was injected directly into the perfusate proximal to the arterial cannula at 5 minute intervals. Once stable responses to PNS and NA infusion had been demonstrated, perfusion with other drugs began. The method of perfusion of the drugs was similar to that described previously in Chapter 2 for the isolated mesenteric vasculature. Once again the second response was taken for all the responses elicited by the PNS and NA infusion; the exception being experiments involving cocaine.

PNS and NA response data after drug perfusion in the preparations are expressed as a percentage of the control pressor response in order to standardize the data.
Male and female Japanese Hypertensive (SH) rats (Okamoto and Aoki, 1963) and female New Zealand Hypertensive rats (NZH) (Phelan, E.L., 1968) were compared to age matched male and female Wistar rats (University of Bath strain) and female New Zealand Normotensive rats (NZN) (University of Bath strain), respectively. The animals used were 14-16 weeks old and were supplied by the University of Bath Animal House.

Systolic blood pressure was monitored in the conscious animal by the tail-cuff method using a programmed electro-sphygmomanometer PE-300 (Narco bio-instruments) coupled to a flat-bed recorder (CR-6505, J J instruments).

3.2ii Statistical Analysis

Results were analysed using Student's t-test for group and paired mean comparisons. Probability levels equal to or less than 0.05 were taken as indicating statistically significant differences. All comparisons employed the two-tailed test.

3.3 Results

3.3i Table 3.1 summarises the systolic blood pressure, basal perfusion pressure and the pressor response to PNS and NA infusion in the six groups of rats.

It can be seen that in addition to having a significantly higher blood pressure than the normotensive rats, the pressor responses to PNS and to NA infusion were also significantly greater in the SH and NZH preparations. The male rats generally had a higher basal perfusion pressure than the females ($p<0.01$ for the SH rats and $p<0.001$ for the Wistars). After the experiments described below (usually one hour), the weights of the kidneys were noted in order to ascertain the degree of oedema formation. As can be seen in Table 3.2, the degree of oedema formation was kept to a minimum and its formation was essentially the same in the preparations from all the animals used this study.

3.3ii Effect of isoprenaline on the pressor response to PNS and NA infusion

Isoprenaline alone had no significant effect on the basal perfusion pressure of the isolated kidney. In the preparations from the SH and NZH rats, isoprenaline (10^{-10}M to 10^{-7}M) caused a dose-dependant potentiation of the pressor response to PNS. At higher isoprenaline

Table 3.1

Systolic blood pressure and basal perfusion pressure, pressor responses to PNS and NA infusion in kidneys from male and female SH (SHR_m and SHR_f, respectively) male and female Wistar (W_m and W_f, respectively) and female NZH and NZN rats

	Systolic blood pressure (mm Hg)	Basal perfusion (mm Hg)	Pressor Response	
			PNS (3Hz, 50v, 1msec for 5 sec (mm Hg)	NA infusion (0.1 of 10^{-7}M) (mm Hg)
SHR_m	. 191 ± 4.5 (20)	186 ± 6.1 (20)	+ 25.25 ± 2.1 (10)	* 18.5 ± 0.8 (10)
W_m	95 ± 5 (20)	189 ± 4.0 (20)	17.75 ± 1.0 (10)	9.4 ± 0.5 (10)
SHR_f	. 183 ± 5 (20)	150 ± 7.3 (20)	+ 25.85 ± 3.4 (10)	* 19.3 ± 1.0 (10)
W_f	96 ± 4 (20)	155 ± 7.5 (20)	16.25 ± 1.2 (10)	10.9 ± 0.8 (10)
NZH	. 180 ± 5 (20)	151.5 ± 4.7 (20)	+ 22 ± 2.1 (10)	* 18.3 ± 0.7 (10)
NZN	94.2 ± 4 (20)	149.5 ± 4.4 (20)	17 ± 0.9 (10)	9.2 ± 0.6 (10)

Values are given as mean ± s.e of the mean.
Number in parenthesis = number of animals
. indicates significant differences between SH, NZH rats and respective control rats ($p<0.01$)
+ indicates significant differences between SH, NZH rats and respective control rats ($p<0.05$)
Basal perfusion pressure significantly greater in male rats than in female rats ($p<0.01$)
* indicates significant differences between SH,NZH rats and respective control rats ($p<0.001$)

Table 3.2

Wet weight of kidneys and oedema formation in the kidneys of male and female SH and Wistar rats (SH_m, SH_f, W_m and W_f respectively) and female NZH and NZN rats

	% change in wet weight	Wet weight (g) of kidney before start of experiment
SHR_m	105 ± 1.0	1.32 ± 0.02
W_m	107 ± 1.1	1.32 ± 0.03
SHR_f	105 ± 0.9	1.23 ± 0.02
W_f	105 ± 0.7	1.22 ± 0.01
NZH	105 ± 0.4	1.25 ± 0.02
NZN	106 ± 0.8	1.24 ± 0.02

Values are mean ± s.e of mean
n = 10 kidney for each group
Wet weight of kidney significantly greater in males than in females ($p<0.01$)

concentrations, around 10^{-6}M, no potentiation of the PNS pressor response was observed. Figs 3.1 a,b,c.

At lower isoprenaline concentration (10^{-11}M to 10^{-8}M) a smaller potentiation of the PNS pressor response was observed whereas the higher isoprenaline concentration (10^{-7}M to 10^{-6}M) caused an inhibition of the vasoconstrictor response to PNS in the preparations from the normotensive animals.

A typical trace of the potentiating effect of isoprenaline on the PNS response in the preparation from the SH rat is shown in Fig 3.2

Significant differences in the degree of potentiation were observed between the hypertensive and respective normotensive animals at each concentration of the isoprenaline.

As can be seen from Figs 3.3 a,b,c isoprenaline caused a dose dependant inhibition of the pressor response to NA infusion in the preparations from both, the hypertensive and the normotensive animals. There was no significant difference in the degree of inhibition between the preparations from the hypertensive and respective normotensive rats.

In order to investigate the effects of β-adrenoreceptor antagonists in the preparation from the various groups, a

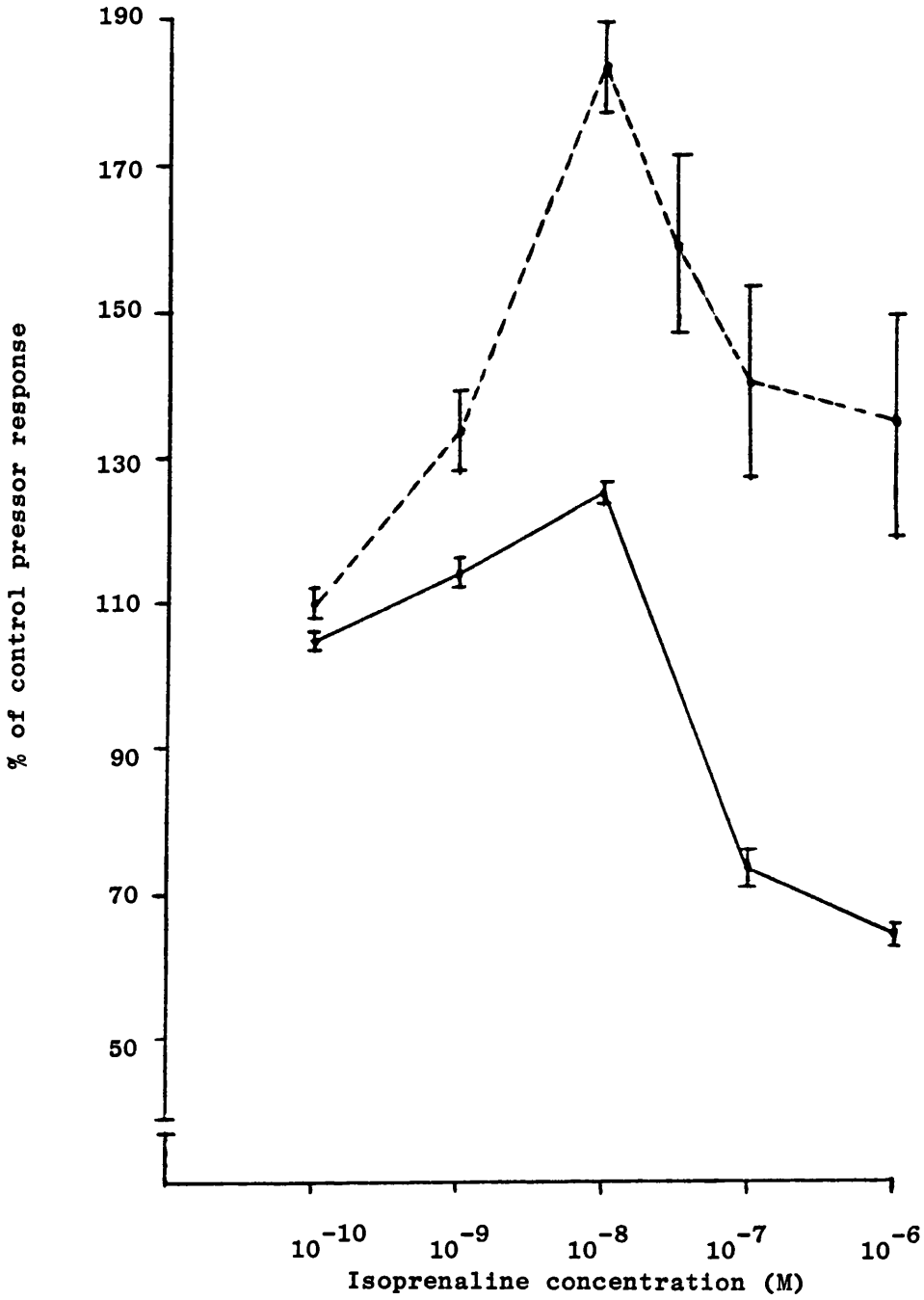


Fig 3.1a Effect of isoprenaline on the pressor response to PNS in the isolated perfused kidneys from male SH (•---•) and male Wistar (•——•) rats.
Vertical lines indicate s.e. of mean.n = 4 animals for each group
At all points, SH response significantly greater than Wistar response ($p<0.05$)

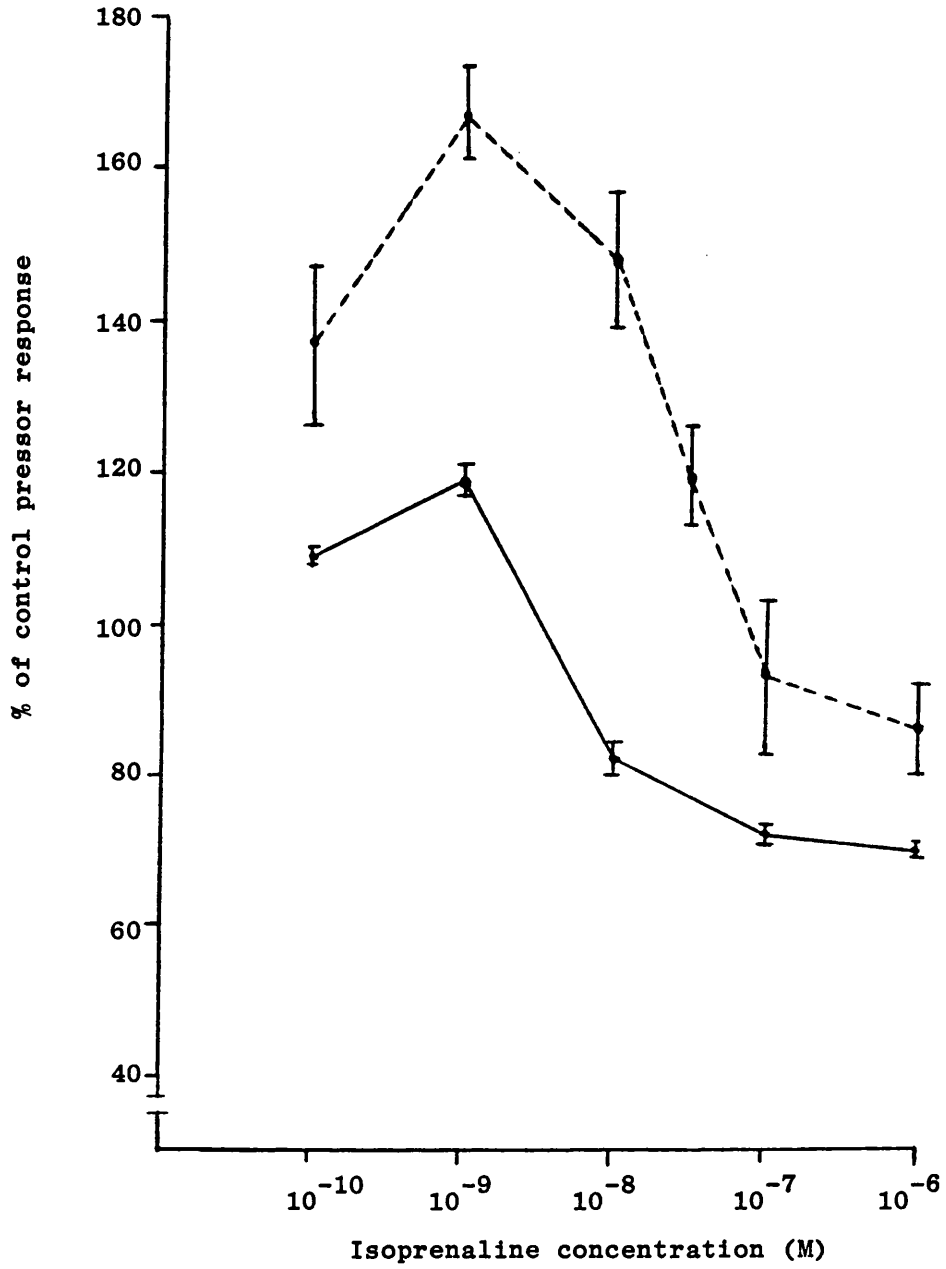


Fig 3.1b Effect of isoprenaline on the pressor response to PNS in the isolated perfused kidneys from female SH (•----•) and female Wistar (•——•) rats.
Vertical lines indicate s.e. of mean, n = 6 animals for SH rats, n = 4 animals for Wistar
At all points, SH rat responses significantly greater than in Wistar rat ($p<0.05$)

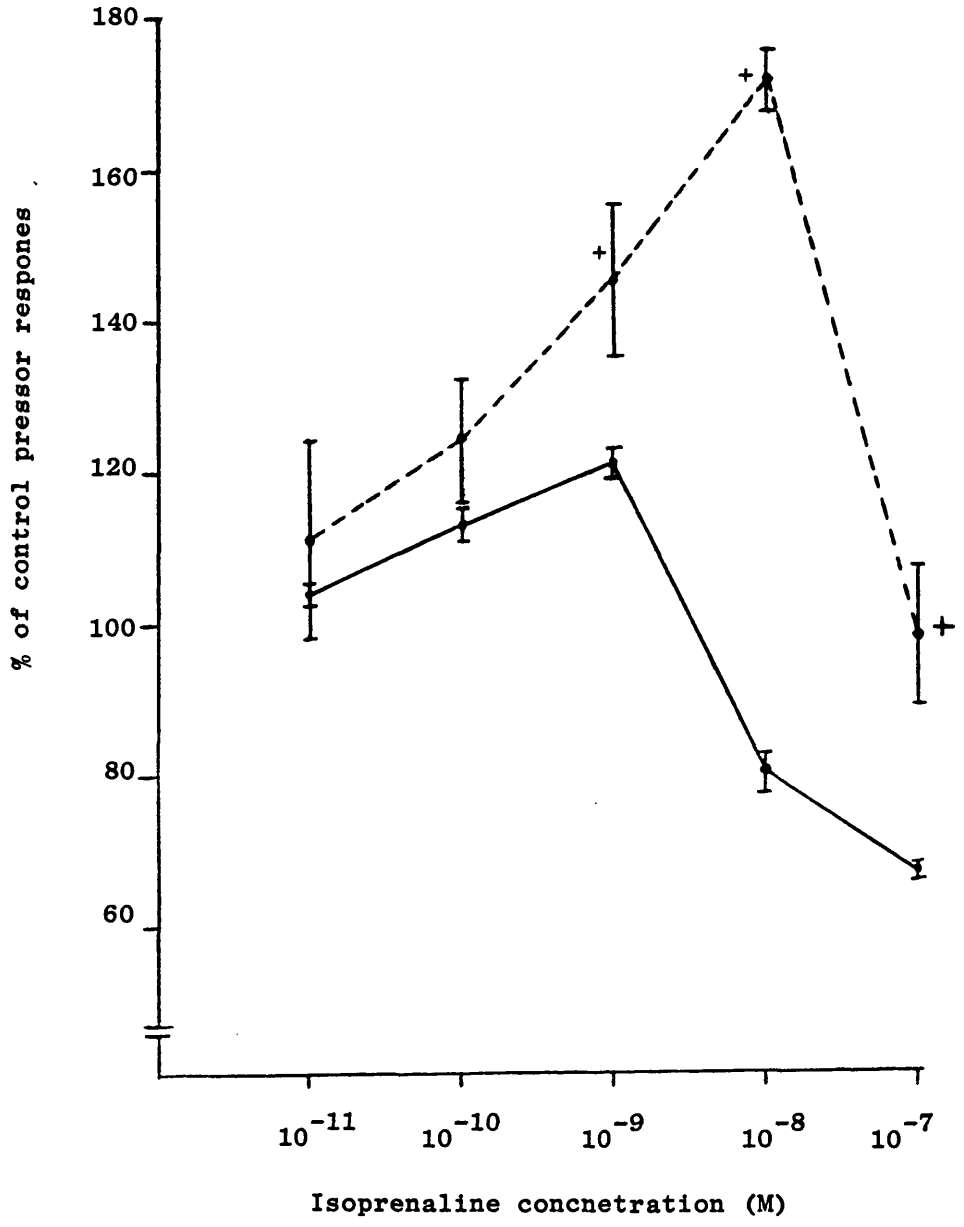


Fig 3.1c Effect of isoprenaline on the pressor response to PNS in the isolated perfused kidneys of female NZH (•---•) and female NZN (•——•) rats. Vertical lines indicate s.e. of mean. n = 5 animals for NZH rats, n = 4 animals for NZN rats.

\+ indicates significant differences between NZH and NZN responses ($p<0.05$)

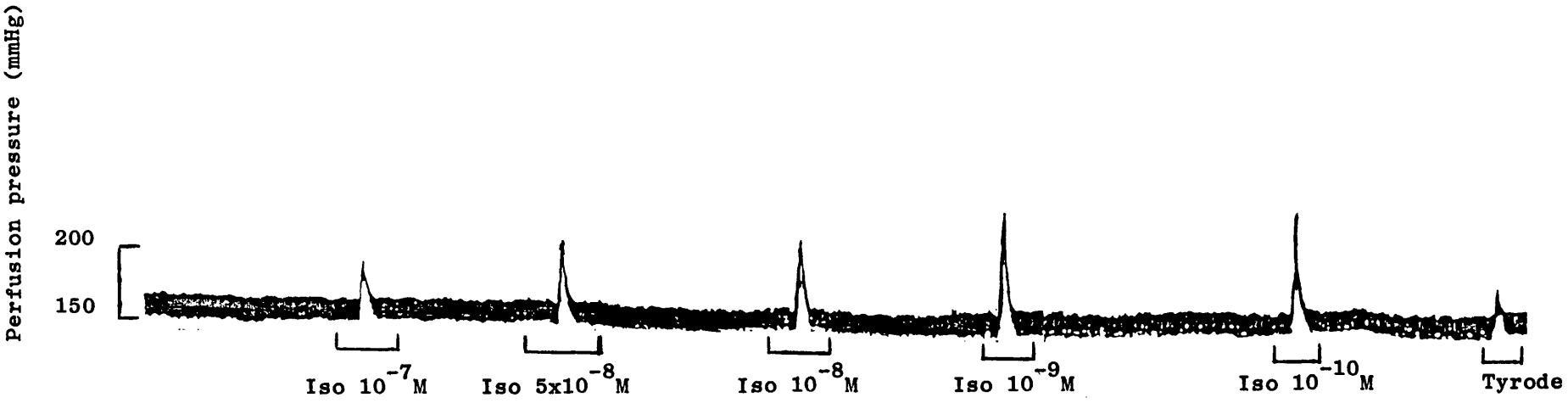


Fig 3.2. Typical trace of the facilitatory effect of isoprenaline (Iso) on the pressor response to peri arterial nerve stimulation in the isolated perfused kidney from female SH rat.

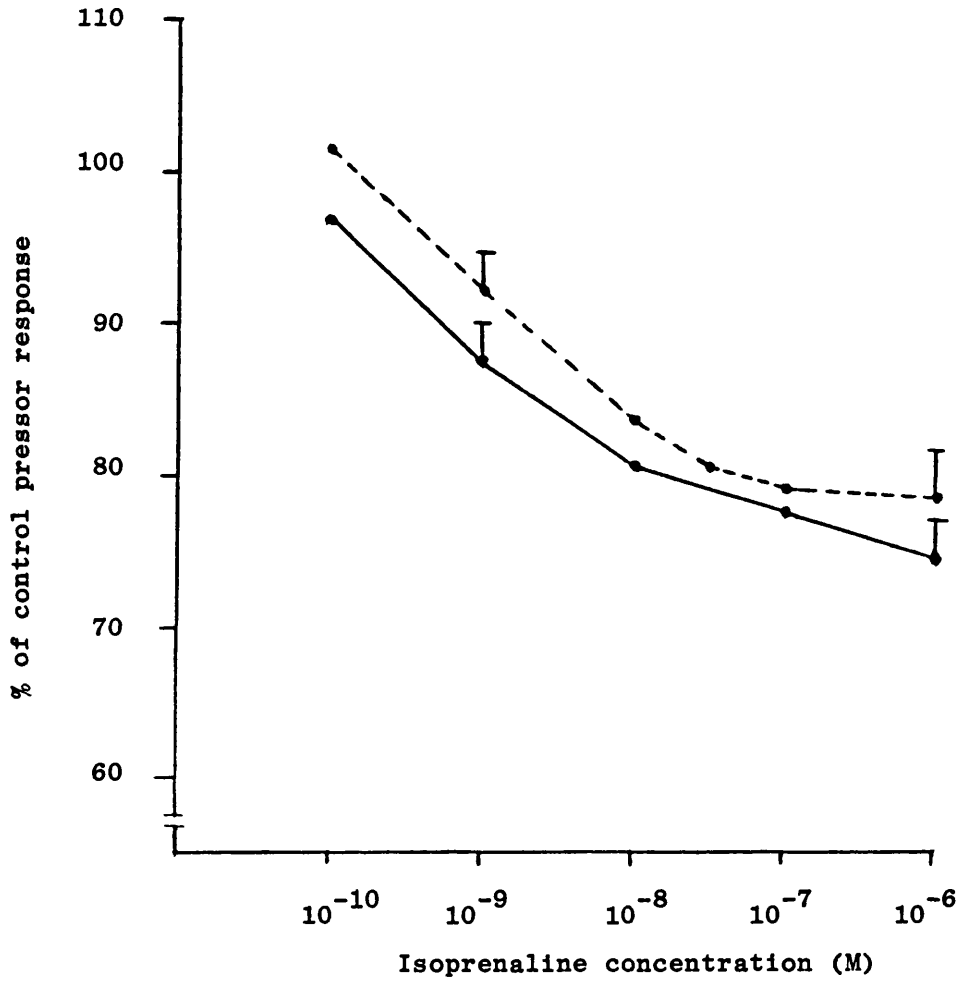


Fig 3.3a. Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused kidneys of male SH (•---•) and male Wistar (•——•) rats.
Vertical lines indicate s.e. of mean (most omitted for clarity). n = 3 animals for each group
Responses from 10^{-9} M to 10^{-6} M isoprenaline significantly different from control Tyrode response ($p<0.05$) for both groups.

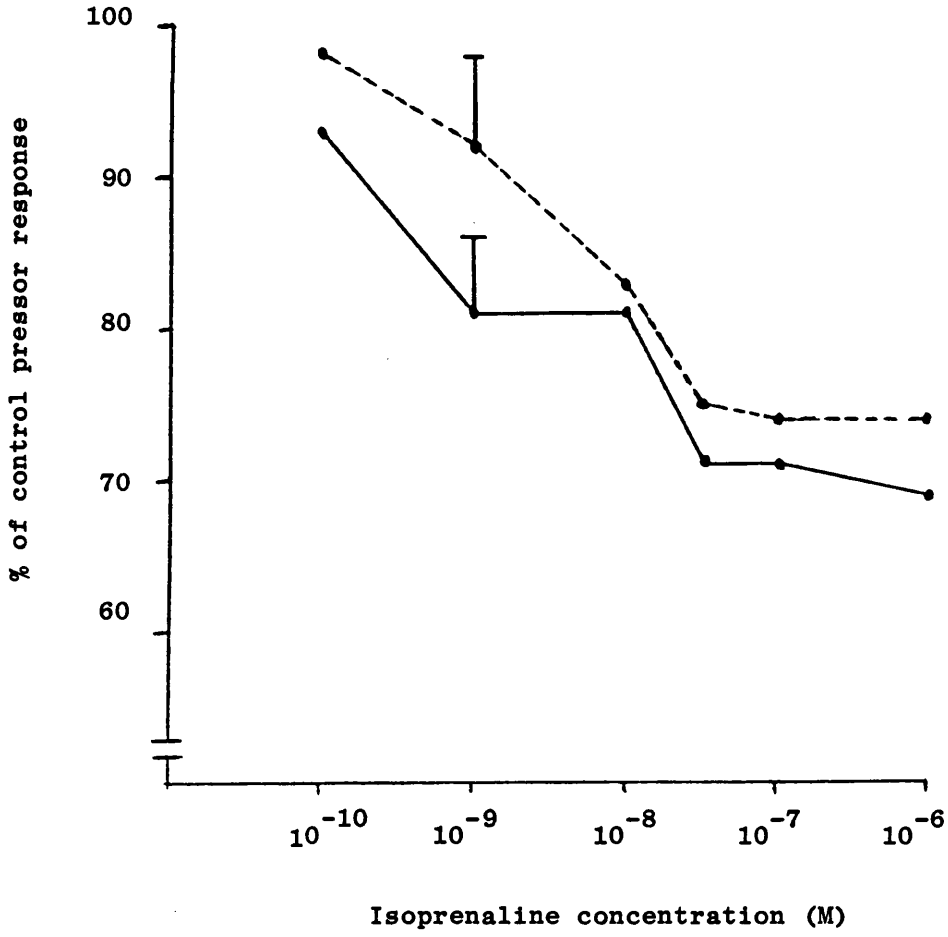


Fig 3.3b Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused kidneys of female SH (●---●) and female Wistar (●——●) rats. Vertical lines indicate s.e. of mean (most omitted for clarity). n = 3 animals for each group.
Responses from 10^{-8} M to 10^{-6} M isoprenaline concentrations significantly different from control Tyrode response ($p<0.05$) for female SH rat.
Responses from 10^{-9} M to 10^{-6} M isoprenaline concentrations significantly different from control Tyrode response ($p<0.05$) for female Wistar rat.

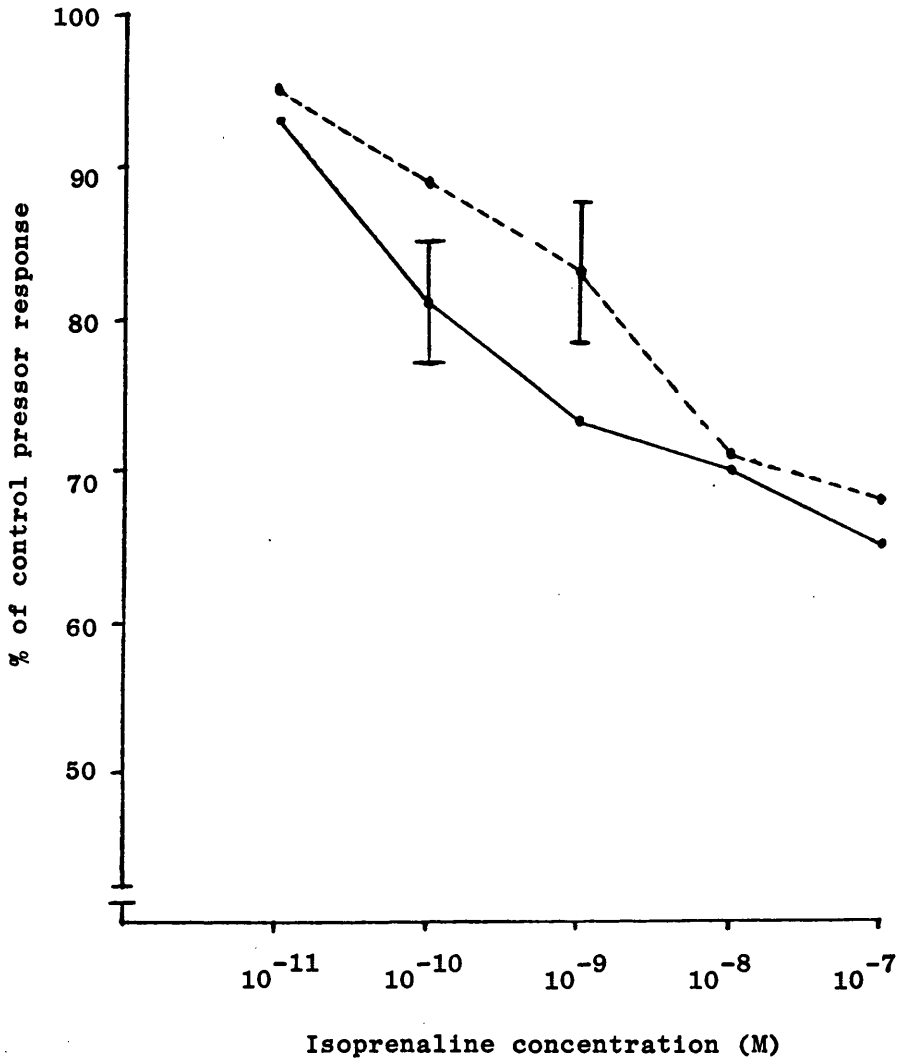


Fig 3.3c. Effect of isoprenaline on the pressor response to NA infusion in the isolated perfused kidneys of female NZH (•---•) and female NZN (•——•) rats.
Vertical lines indicate s.e. of mean (most omitted for clarity).
n = 3 animals for each group.
Responses from 10^{-10} M to 10^{-7} M isoprenaline concentrations significantly different from control Tyrode response ($p<0.05$) for both groups.

standard concentration of isoprenaline was chosen. The standard concentration used was 10^{-9}M in the females and 10^{-8}M in the males as these concentrations caused the greatest potentiation of the PNS pressor response in the control animals (Wistars and NZN).

3.3iii Effect of non-selective β-adrenoreceptor antagonist on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Propranolol ($5x10^{-8}$M) was used as the non-selective β-adrenoreceptor antagonist. At the concentration employed in this study propranolol alone had no significant effect on the basal perfusion pressure or the PNS pressor response, but it significantly inhibited the isoprenaline induced potentiation of the pressor response to PNS in all the preparations. Fig 3.4.

Propranolol alone tended to cause a moderate enhancement of the pressor response to NA infusion in the preparation from the male SH rat only. The inhibitory effect of isoprenaline on the pressor response to NA infusion was reversed significantly by propranolol in the preparations from all the groups. Fig 3.5.

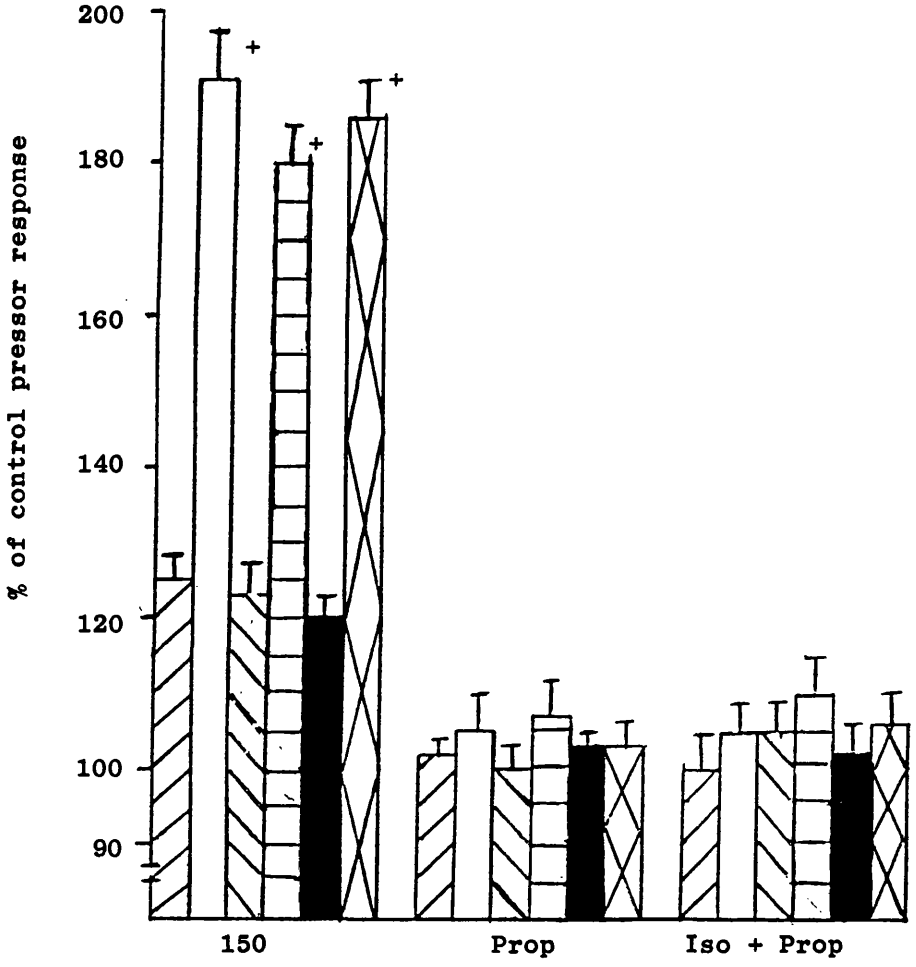


Fig 3.4. Effect of Prop(propranolol) on the isoprenaline (ISO)-induced facilitation of the pressor response to PNS of the isolated perfused kidneys of the SH, Wistar, NZH and NZN rats.

Male SH rat ☐ Female SH rat ▭ Female NZH rat ☒

Male Wistar rat ▨ Female Wistar rat ▧ Female NZN rat ■

Vertical lines indicate s.e. of mean.
n = 4 animals for each group.
All groups in Iso section significantly different from control response and all other sections ($p<0.05$)
+ $p<0.05$ compared to respective normotensive animals.

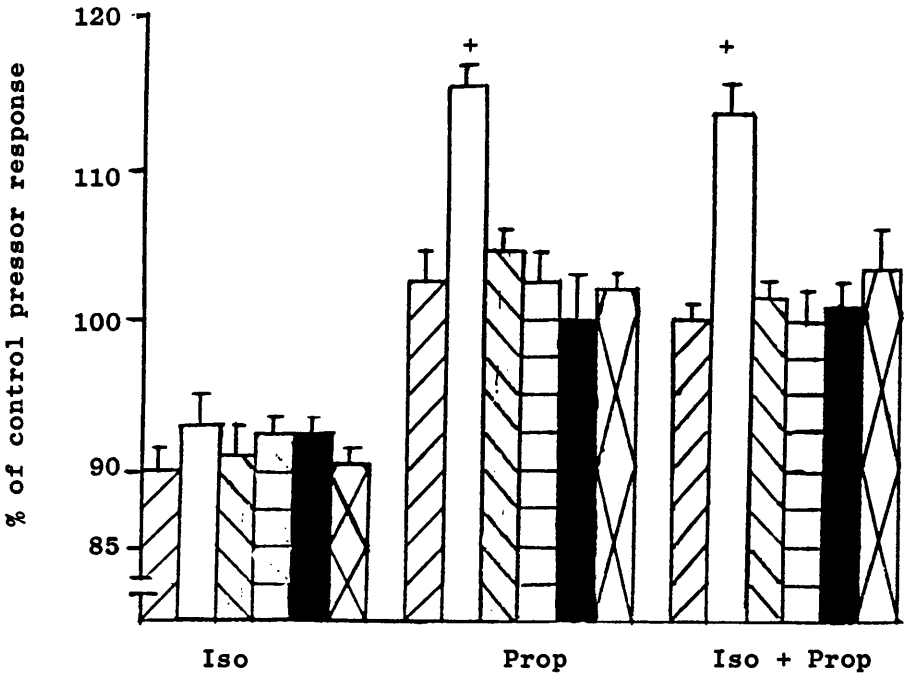


Fig 3.5 Effect of Prop (propranolol) on the Iso (isoprenaline)-Induced inhibition of the pressor response to exogenous NA in the isolated kidney of SH,Wistar, NZH and NZN rats.

Male SH rat ☐ Female SH rat ☐ Female NZH rat ☒

Male Wistar rat ▨ Female Wistar rat ▨ Female NZN rat ■

Vertical lines indicate s.e. of mean
n = 3 animals for each group.
All groups in Iso section significantly different from control response ($p<0.05$)
+ $p<0.05$ compared to control response

3.3iv Effect of selective β_1- adrenoreceptor antagonist on the isoprenaline induced effects on the pressor respones to PNS and NA infusion

Atenolol (10^{-7}M) was used as the selective β_1 - adrenoreceptor antagonist. At the concentration employed in this study, atenolol had no significant effect on the basal perfusion pressure or the pressor response to PNS in any of the preparations. Atenolol did not inhibit the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations from any of the animals. Fig 3.6.

Atenolol (10^{-7}M) alone tended to enhance significantly the pressor response to NA infusion in the preparations from male SH animals only (approximately 16% of control). This enhancement was not observed in any of the preparations from any of the other animals. In all the preparations, the atenolol reversed significantly the isoprenaline induced inhibition of the pressor response to NA infusion. Fig 3.7.

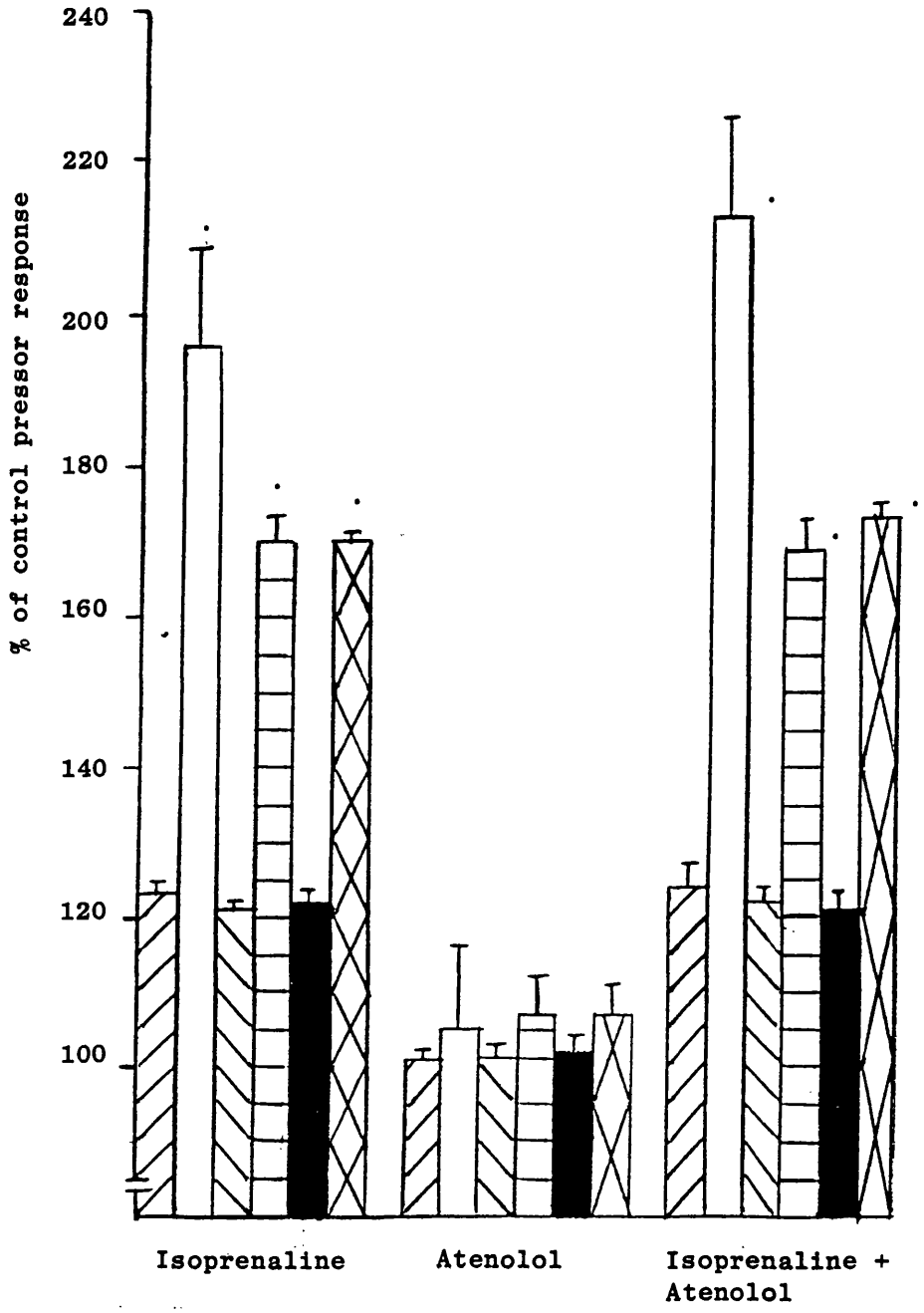


Fig 3.6 Effect of atenolol on the isoprenaline induced potentiation of the pressor response to PNS in the isolated perfused kidney of SH, Wistar, NZH and NZN rats.

Male SH rat ☐ Female SH rat ▭ Female NZH rat ☒

Male Wistar rat ▨ Female Wistar rat ▧ Female NZN rat ■

Vertical lines indicate s.e. of mean.
n = 3 animals for each male group, n = 4 animals for each female group.
All groups in Isoprenaline section and Isoprenaline + atenolol section $p<0.05$, compared to control response.
. $p<0.05$ compared to respective normotensive animal.

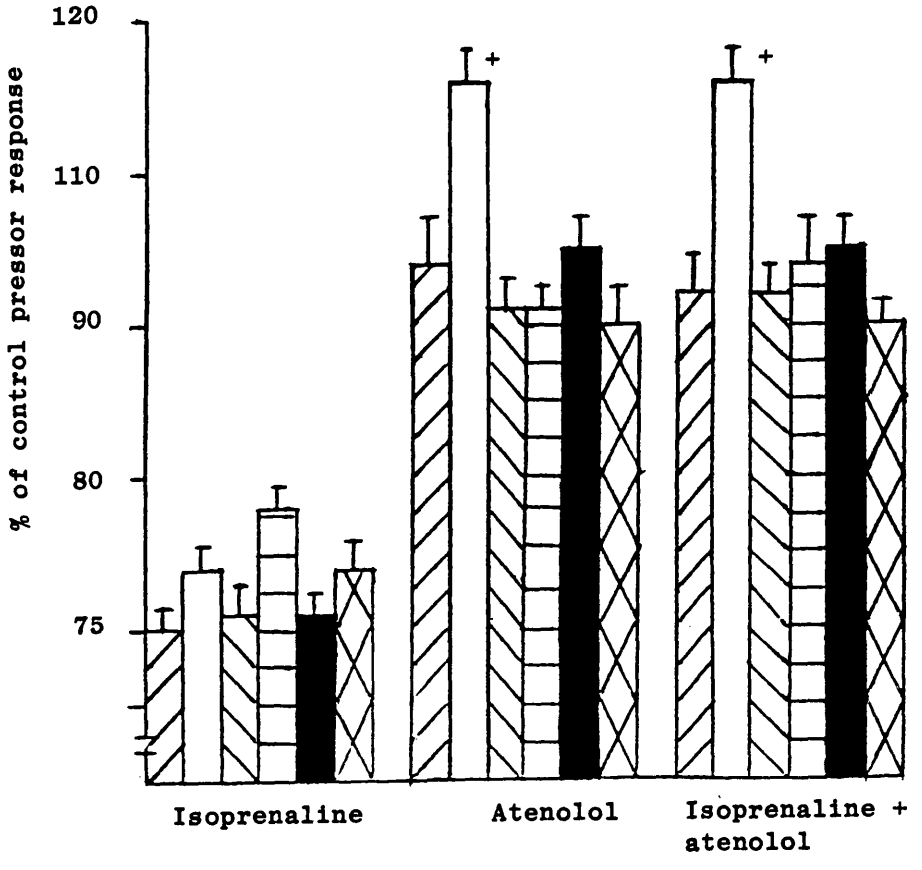


Fig 3.7. Effect of atenolol on the isoprenaline-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidneys of SH, Wistar NZH and NZN rats.

Male SH rats ☐ Female SH rats ▤ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
All groups in isoprenaline section, $p<0.05$, compared to control response and all other sections.
+ $p<0.05$ compared to control response.

3.3v Effect of selective β_2-adrenoreceptor antagonist on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

ICI 118,551 ($5x10^{-7}M$) was used as the selective β_2-adrenoreceptor antagonist. At the concentration employed in this study, ICI 118,551 had no significant effect on the basal perfusion pressure or the pressor responses to PNS in any of the preparations. ICI 118,551 however, inhibited significantly the isoprenaline induced potentiation of the pressor response to PNS in the preparations from all the animals. Fig 3.8.

ICI 118,551 ($5x10^{-7}M$) also reversed significantly the isoprenaline induced inhibition of the pressor response to NA infusion in the preparations from all the animals. Fig 3.9.

3.3vi Effect of angiotensin II on the pressor response to PNS and NA infusion

Angiotensin II (1 ng/ml) was found to cause a transient elevation of the basal perfusion pressure by around 20% when it was first perfused in the preparations. This elevation usually lasted for around 90 seconds before the perfusion pressure gradually returned to its pre-angiotensin II perfusion levels. Further perfusion of angiotensin II in the same preparation caused no

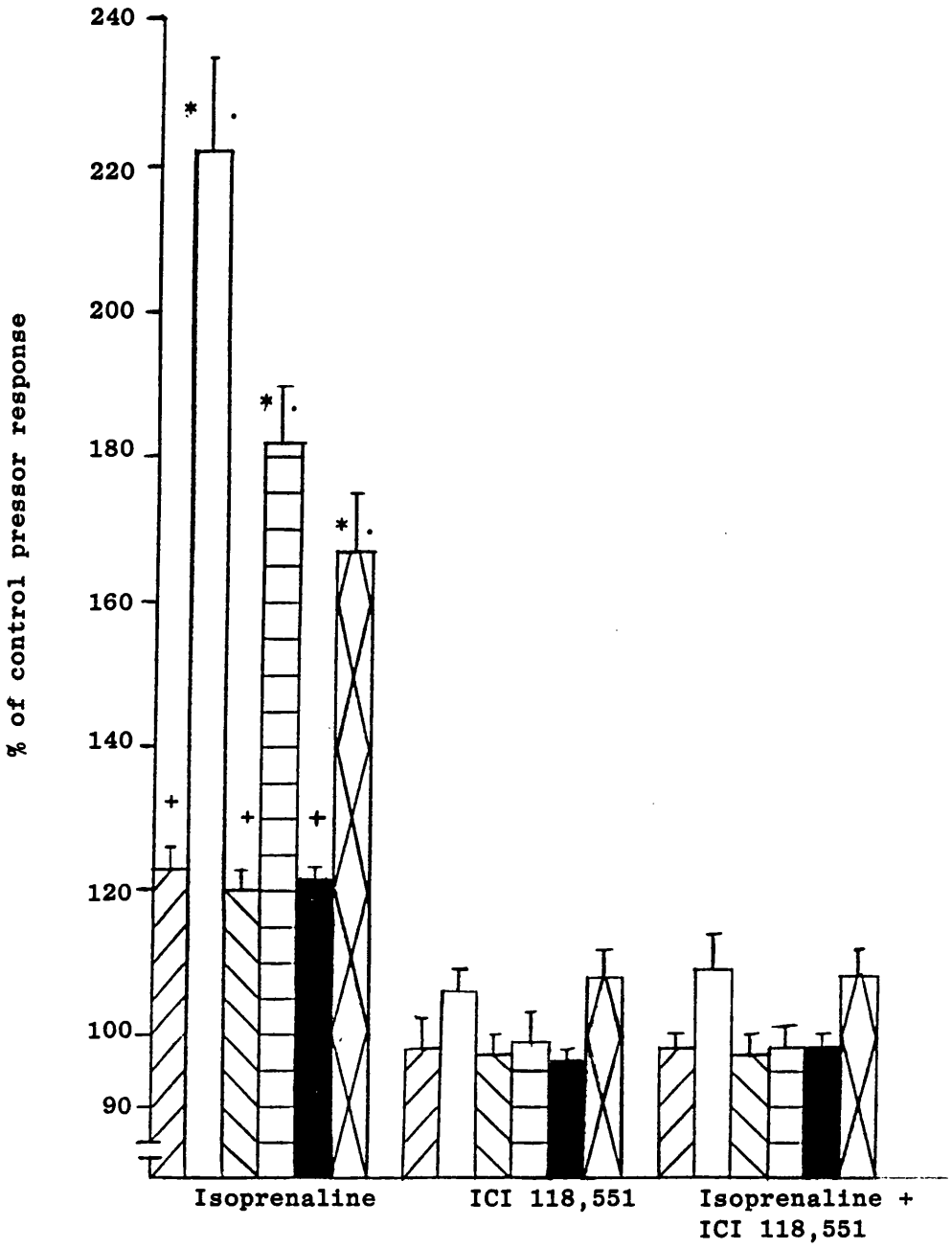


Fig 3.8. Effect of ICI 118,551 on the isoprenaline-induced potentiation of the pressor response to PNS in the isolated perfused kidneys of SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ▤ Female NZH rats ▩

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each male group and n = 4 animals for each female group.
+ $p<0.05$, * $p<0.01$ compared to control response.
. $p<0.05$ compared to respective normotensive animal.

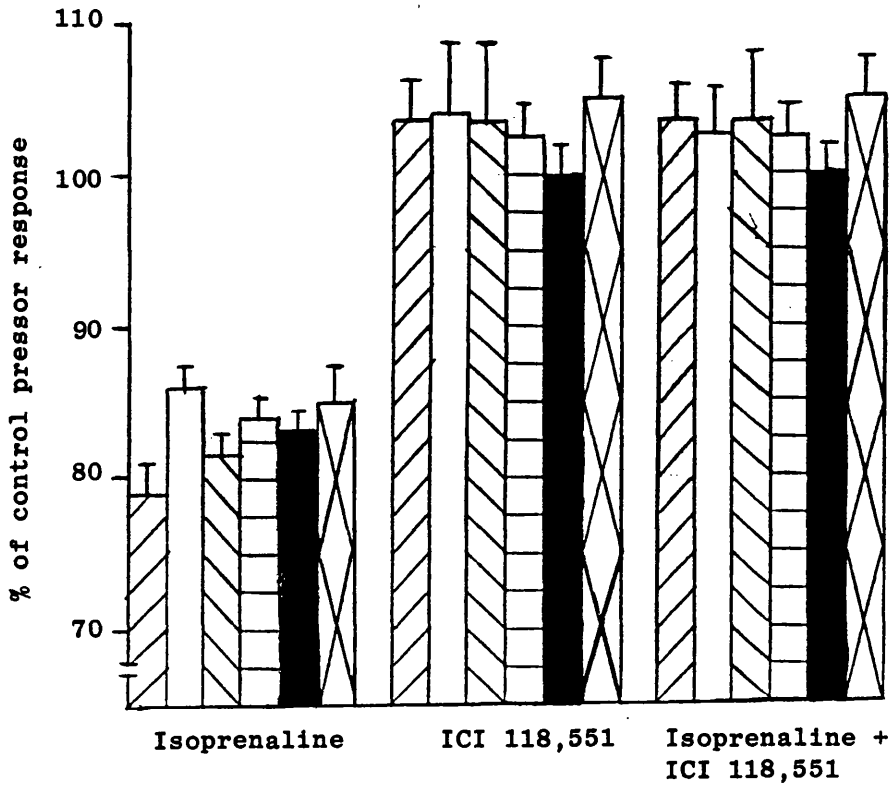


Fig 3.9 Effect of ICI 118,551 on the isoprenaline-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidneys of SH, Wistar NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ⊠

Male Wistar rats ⧄ Female Wistar rats ⧅ Female NZN rats ■

Vertical lines indicate s.e. of mean n = 3 animals for each group.
All groups in isoprenaline section, $p < 0.05$, compared to other groups.

discernable effect on the basal perfusion pressure. Angiotensin II significantly potentiated the pressor response to both PNS and NA infusions in all the preparations. Fig 3.10. The potentiating effect of angiotensin II on the PNS pressor response was found to be significantly greater in the preparations from SH and NZH animals than that in their respective controls.

The degree of the potentiating action of angiotensin II on the pressor response to NA infusion was found to be generally similar in all the preparations. Fig 3.11.
In the preparations from the normotensive animals, the degree of potentiation of the pressor response to PNS was similar to that of NA infusion; however in the preparations from the hypertensive animals the degree of potentiation of the pressor response to PNS was significantly greater than that of NA infusion.

The angiotensin II receptor antagonist, [Sar^1-Ile^8] angiotensin II (Sar, 200 ng/ml), which itself did not significantly affect the basal perfusion pressure or the pressor response to PNS and NA infusion, completely prevented the potentiation of the pressor response to PNS and NA infusion induced by the angiotensin II. Table 3.3

The effect of cocaine (500 ng/ml) on the pressor response to PNS and NA infusion is shown in Fig 3.12. and 3.13. The concentration of cocaine employed in this study did not significantly affect the basal perfusion pressure. As can

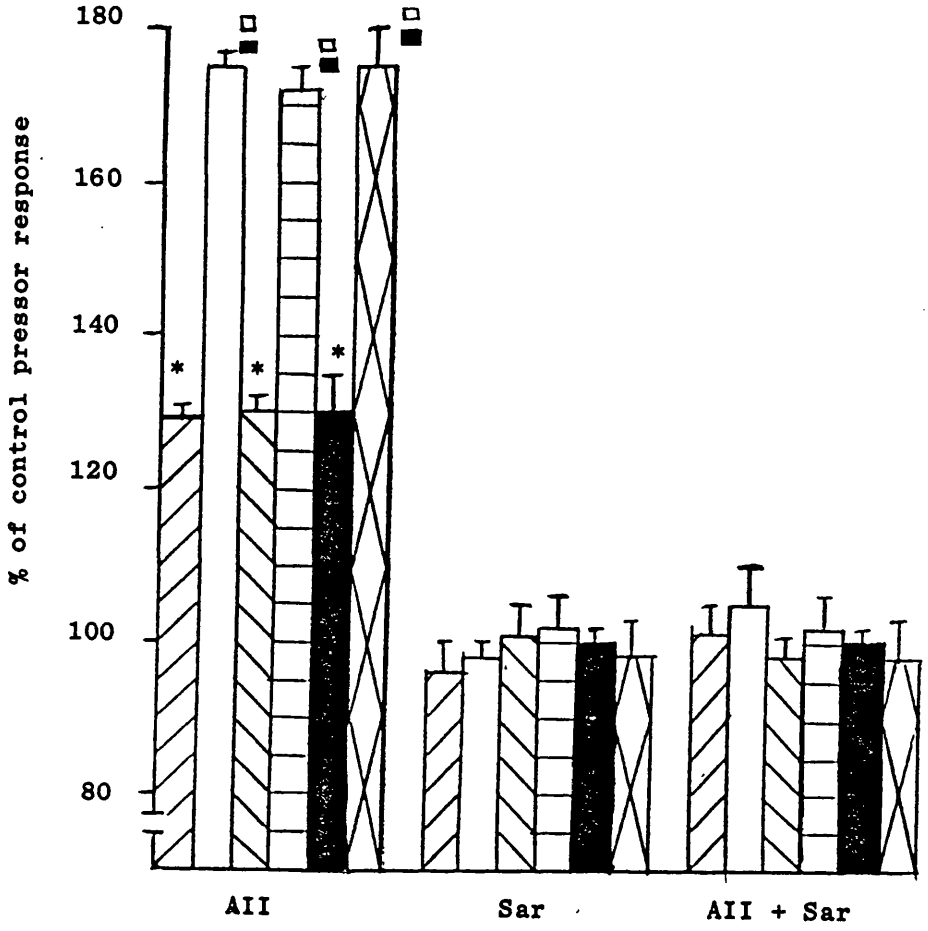


Fig 3.10

Effect of angiotensin II (AII) and [Sar^1-Ile^8] angiotensin II (Sar) on the pressor response to PNS in isolated perfused kidneys from SH, Wistar, NZH and NZN rats.

Male SH rats □ Female SH rats ⊟ Female NZH rats ⊠

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean, n = 4 animals for each group.

* $p<0.01$, □ $p<0.001$ compared to control response and other sections

■ $p<0.01$ compared to respective normotensive rats.

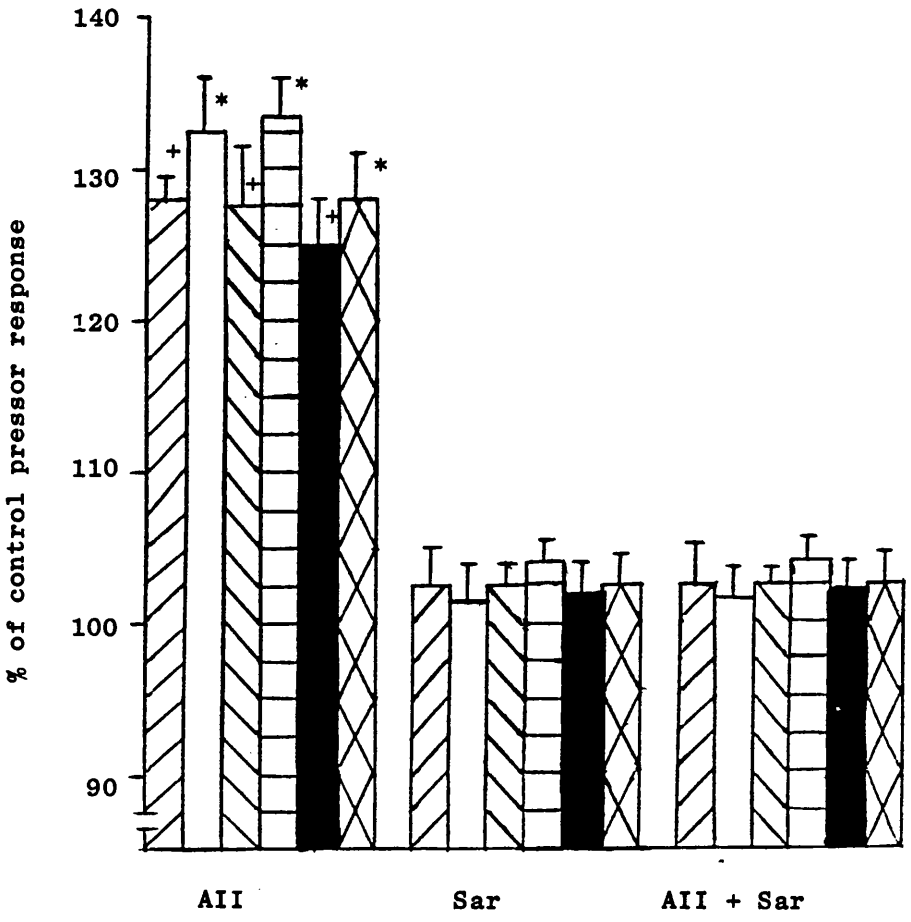


Fig 3.11. Effect of angiotensin II (AII) and [Sar^1-Ile^8] angiotensin II (Sar) on the pressor response to exogenous NA in the isolated perfused kidneys from SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ⊠

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
+ $p<0.05$, * $p<0.01$ compared to control response and to other sections.

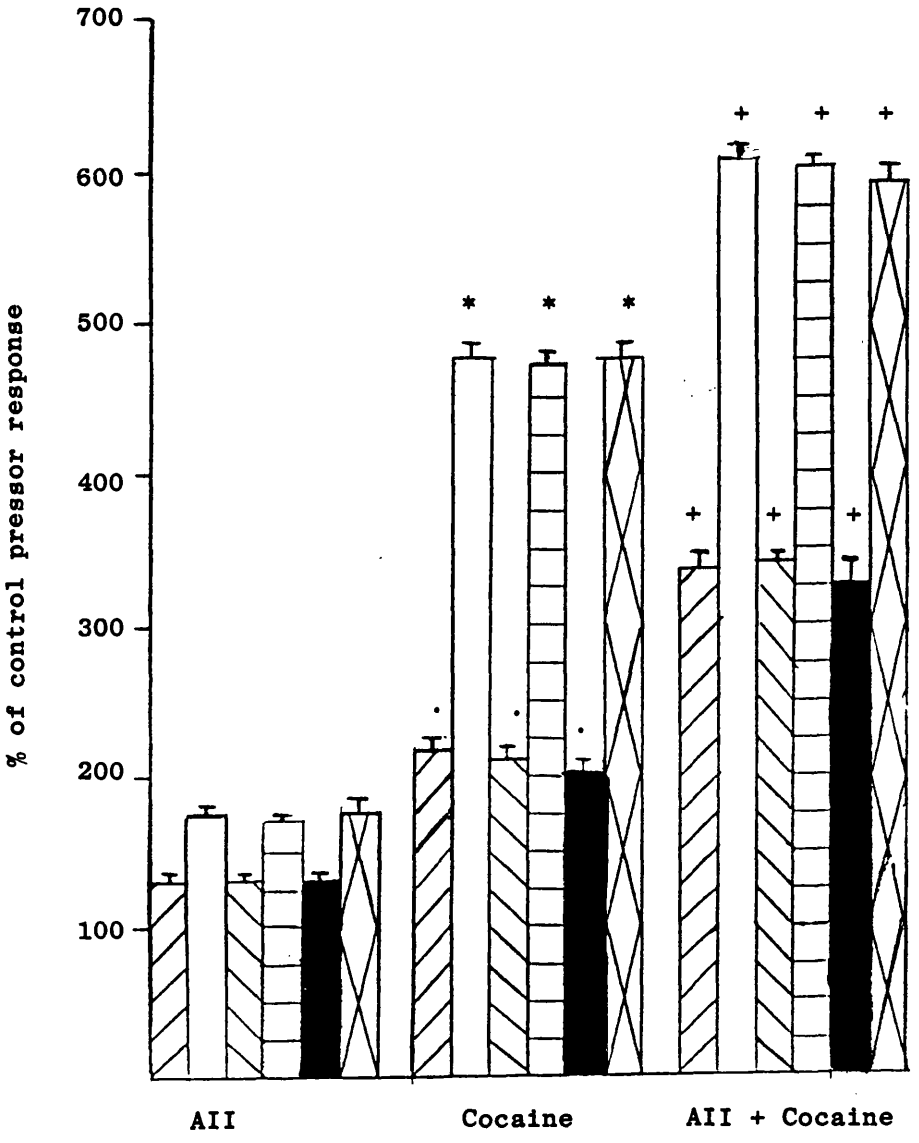


Fig 3.12 Effect of cocaine on the angiotensin II (AII)- induced potentiation of the pressor response to PNS in the isolated perfused kidneys from SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 4 animals for each group.
All sections, hypertensive animals compared to respective normotensive animals, $p<0.01$
+ $p<0.05$ compared to cocaine section
. $p<0.05$, * $p<0.01$ compared to AII section

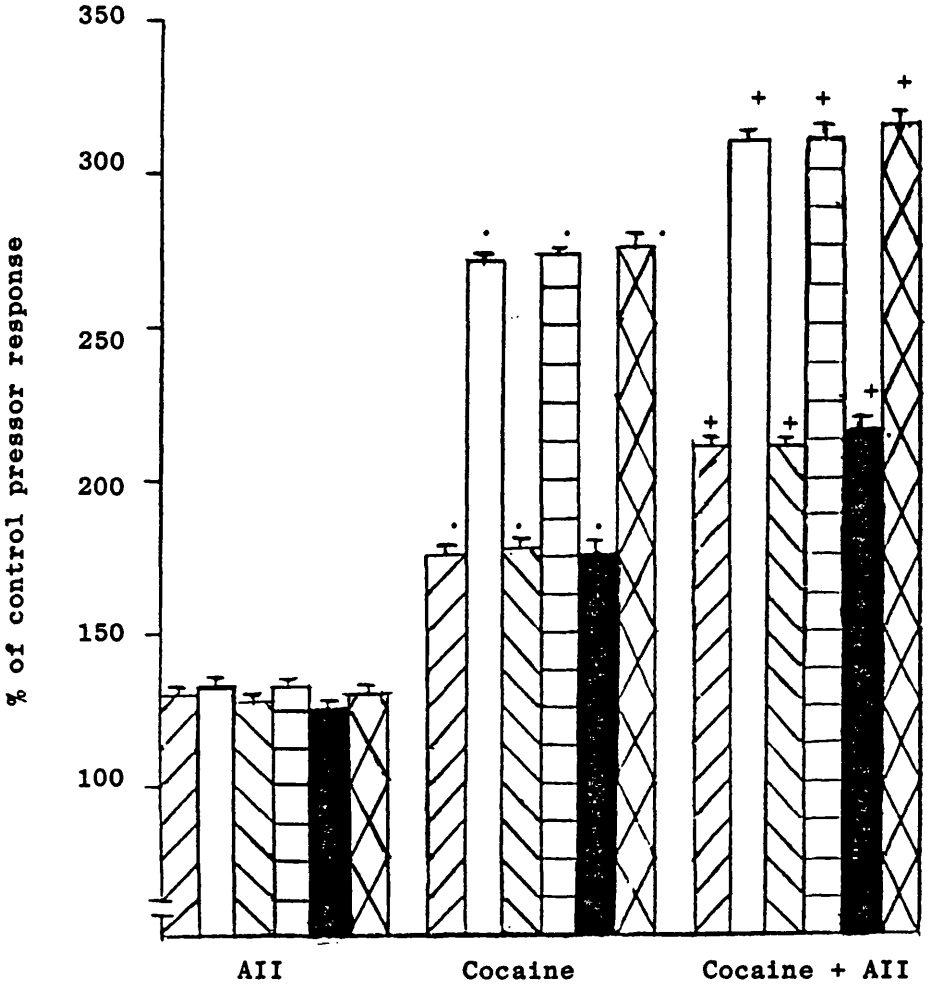


Fig 3.13.

Effect of cocaine and angiotensin II (AII) on the pressor response to exogenous NA in the isolated perfused kidneys of SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ⊠

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean, n = 3 animals for each group.
All sections, except for AII section, hypertensive animals compared to respective normotensive animals, $p<0.05$
+ $p<0.05$ compared to cocaine section
. $p<0.05$ compared to AII section

be seen from Fig 3.12 and 3.13, cocaine significantly potentiated the pressor responses to PNS and NA infusions in all the preparations. The degree of the potentiation of the PNS pressor response was found to be significantly higher than the degree of potentiation of the NA infusion pressor response in all the preparations. The degree of potentiation of the pressor response to PNS and NA infusion was found to be greater in the preparations from the hypertensive animals than that in the respective normotensive animals.

When angiotensin II (1 ng/ml) was simultaneously perfused with the cocaine (500 ng/ml), the potentiation of the pressor response to both PNS and NA infusion was further augmented and the effect appeared to be additive.

The facilitatory effect of angiotensin II on the PNS pressor response was greater in the SH and NZH preparations than in the Wistars and NZN preparations in the presence of cocaine, as well as in its absence. Fig 3.12.

Thus far the results indicate that in the preparations from male and female animals the vasoconstrictor responses to PNS are potentiated by β - adrenoreceptor stimulation and by activation of angiotensin II receptors and that this potentiation is greater in the preparations from SH and NZH animals than that in the Wistars and NZN animals.

The next series of experiments explore the relationship between the β - adrenoreceptors and the angiotensin II receptors.

3.3vii Effect of angiotensin converting enzyme inhibitor on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Captopril ($5x10^{-6}M$) was used as the angiotensin converting enzyme inhibitor in this experiment. At this concentration the captopril had no significant effect on the basal perfusion pressure or the pressor responses to PNS and NA infusion. Captopril, however, effectively inhibited the isoprenaline induced potentiation of the pressor response to PNS in all the preparations, whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion in any of the preparations. Figs 3.14 and 3.15.

3.3viii Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Sar (200 ng/ml) alone had no significant effect on the basal perfusion pressure or pressor responses to PNS and NA infusions. However, Sar effectively inhibited the isoprenaline induced potentiation of the pressor response

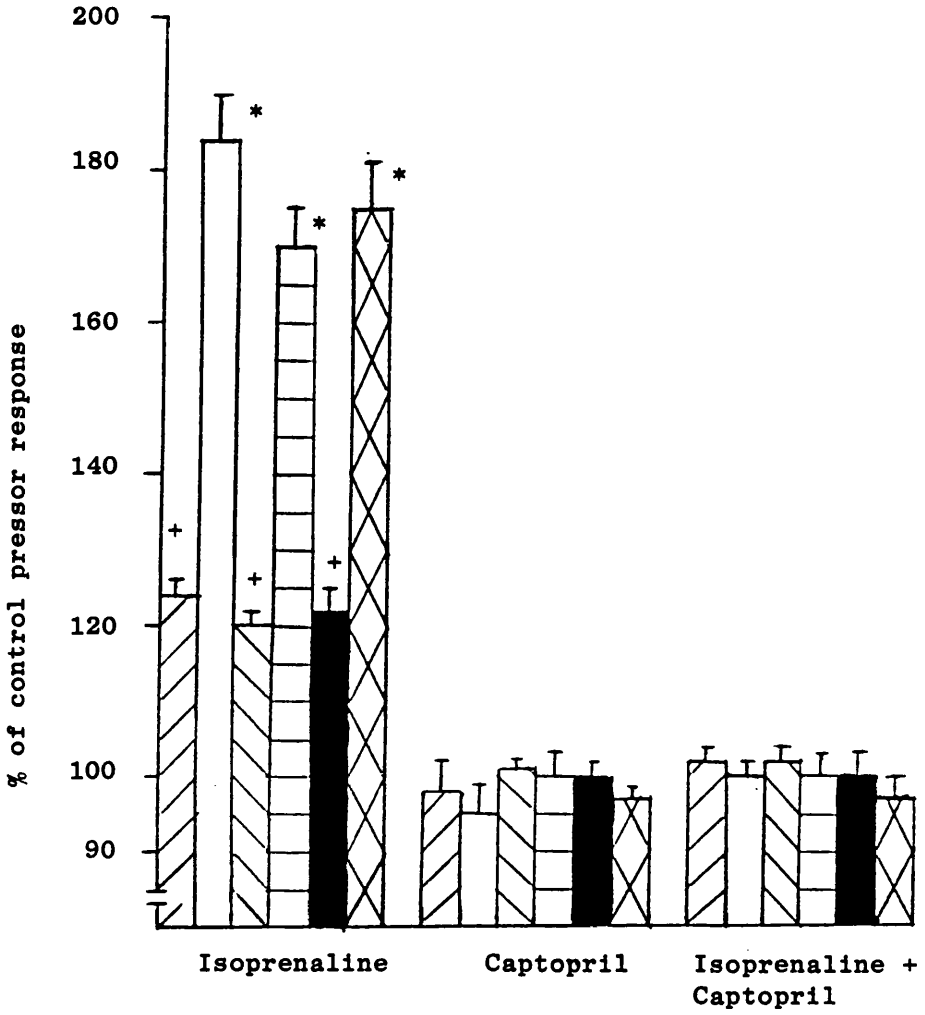


Fig 3.14 Effect of captopril on the isoprenaline-induced potentiation of the pressor response to PNS in the isolated perfused kidneys of SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
+ $p<0.05$, * $p<0.01$ compared to control response and other sections.

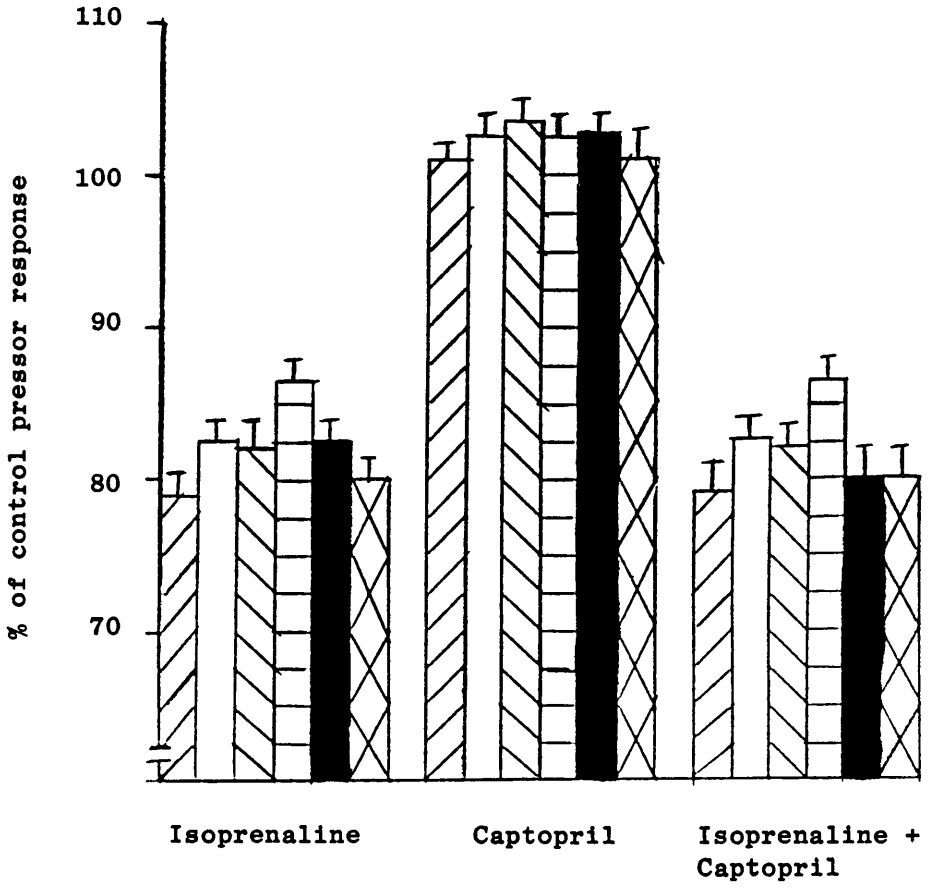


Fig 3.15 Effect of captopril on the isoprenaline-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidney of SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
All groups in isoprenaline section and captopril + isoprenaline section significantly different from control response and captopril section $p<0.05$.

to PNS in all the preparations, whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion in any of the preparations. Figs 3.16 and 3.17.

3.3ix Effect of ICI 118,551 on the angiotensin II potentiation of the pressor response to PNS and NA infusion

As demonstrated earlier, ICI 118,551 ($5x10^{-7}M$) alone had no significant effect on the basal perfusion pressure or pressor responses to PNS and NA infusion. From Fig 3.18 and 3.19 it can be seen that ICI 118,551 had no significant effect on the angiotensin II potentiation of the pressor responses to PNS and NA infusion in any of the preparations.

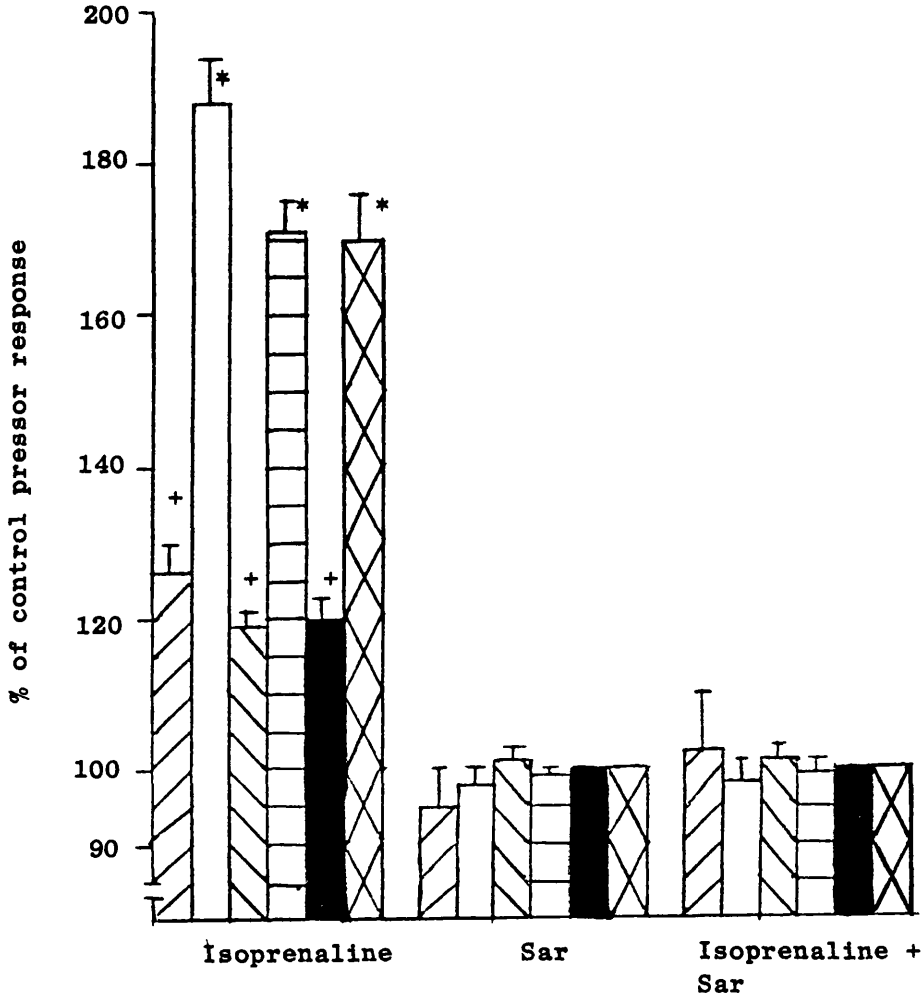


Fig 3.16 Effect of [Sar^1-I-e^8] angiotensin II (Sar) on the isoprenaline-induced potentiation of the pressor response to PNS in the isolated perfused kidneys of SH, Wistar, NZH and NZN rats

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
+ $p < 0.05$, * $p < 0.01$ compared to control response and other sections.

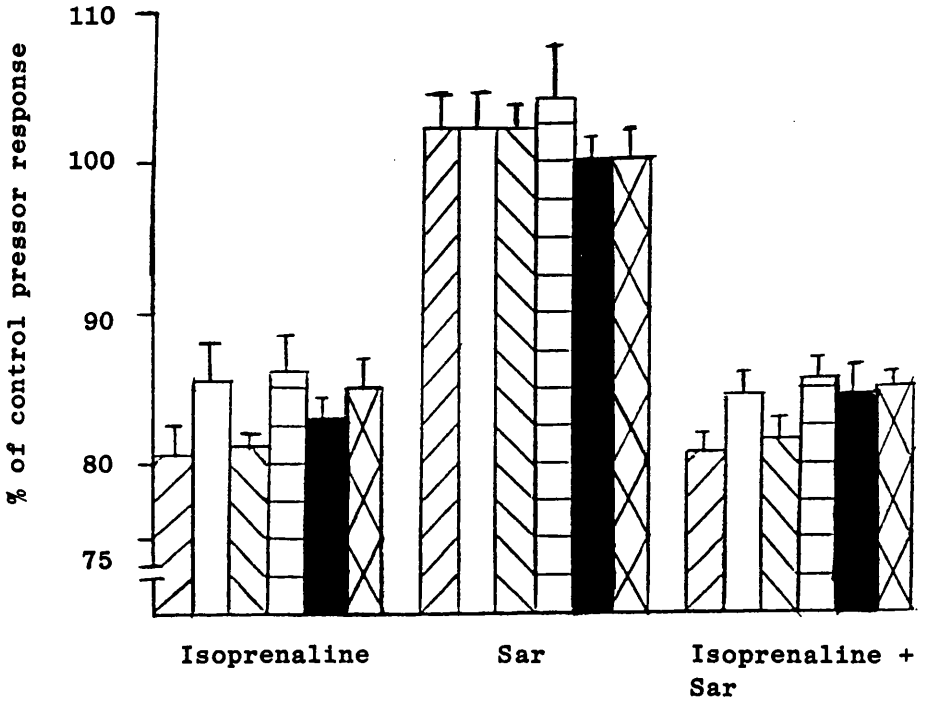


Fig 3.17 Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidneys from SH, Wistar, NZH and NZN rats

Male SH rats ☐ Female SH rats ▤ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
All groups in isoprenaline section and isoprenaline + Sar section significantly different from control response and Sar section $p<0.05$.

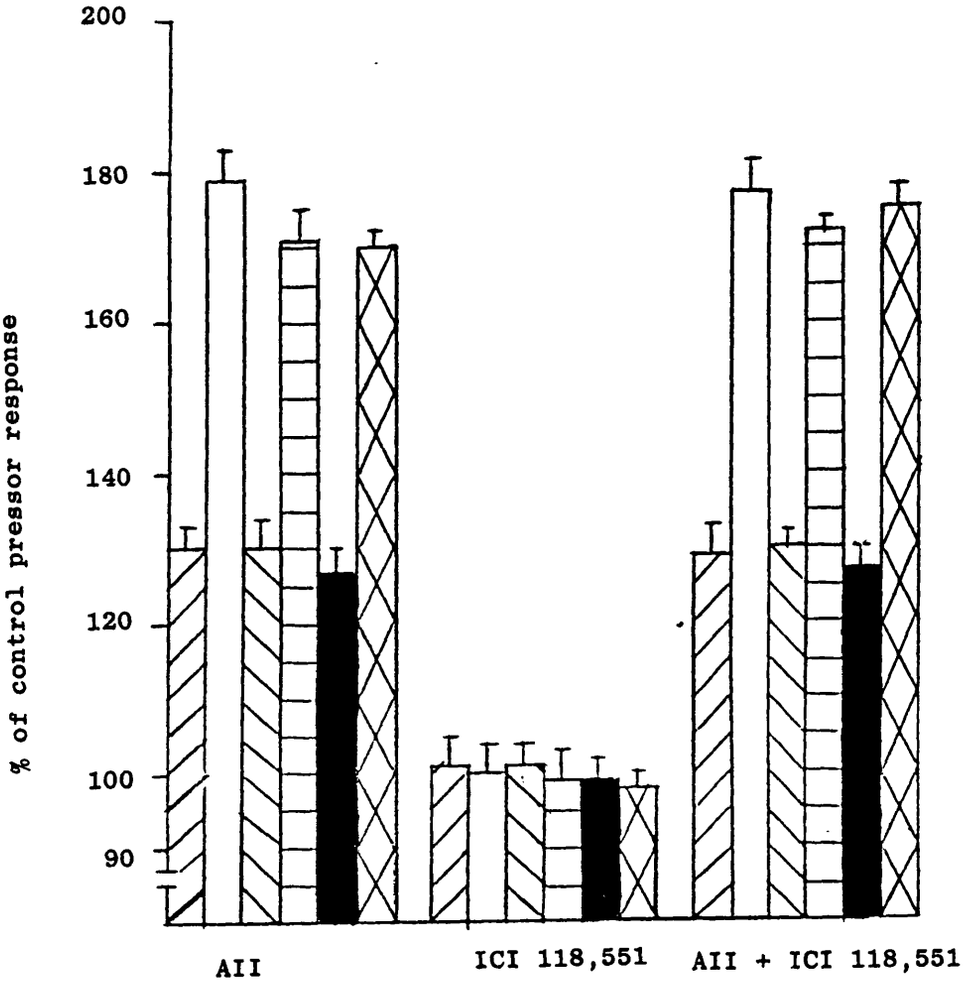


Fig 3.18 Effect of ICI 118,551 on the angiotensin II (AII)-induced potentiation of the pressor response to PNS in isolated perfused kidneys from SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ⧸ Female Wistar rats ⧹ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
ICI 118,551 section significantly different from other sections $p < 0.05$

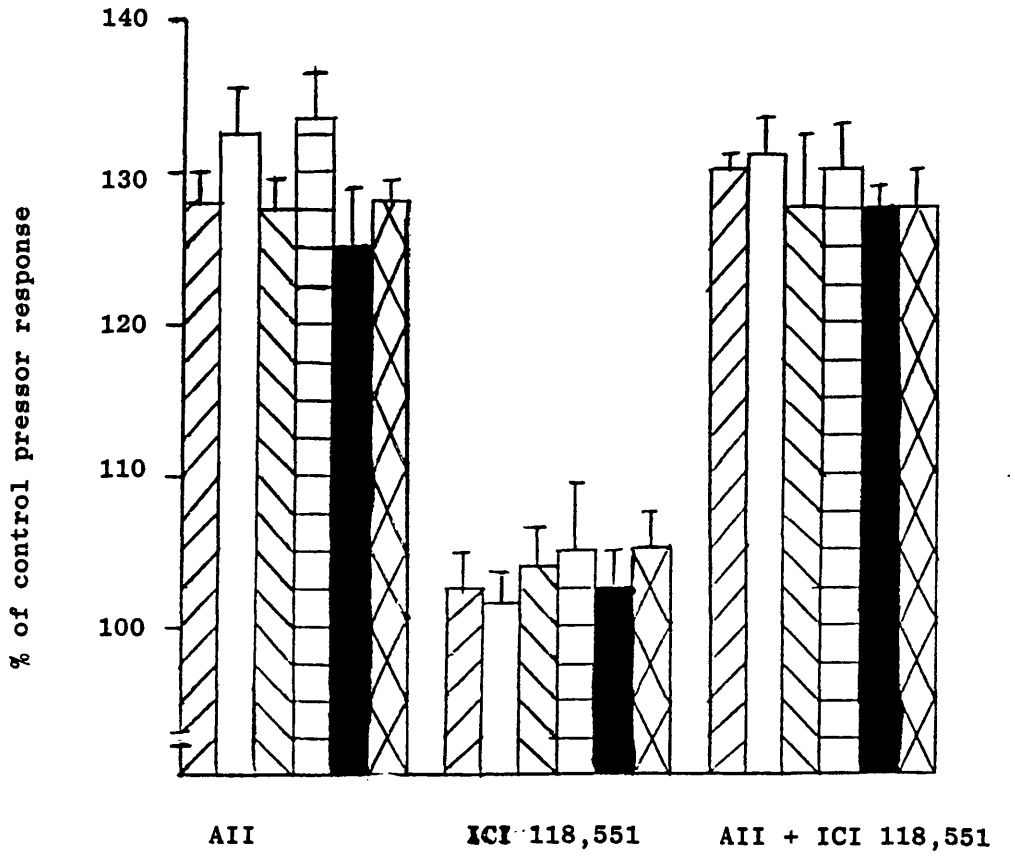


Fig 3.19 Effect of ICI 118,551 on the angiotensin II (AII)-induced potentiation of the pressor response to exogenous NA in the isolated perfused kidneys of SH, Wistar, NZH and NZN rats.

Male SH rats ☐ Female SH rats ⊟ Female NZH rats ☒

Male Wistar rats ▨ Female Wistar rats ▧ Female NZN rats ■

Vertical lines indicate s.e. of mean. n = 3 animals for each group.
ICI 118,551 section significantly different from other sections $p<0.05$.

3.4 Discussion

The results described in this chapter suggest that physiological mechanisms facilitating sympathetic neurotransmission in the kidney are enhanced in the SH and NZH animals (hypertensive animals) compared to the Wistars and NZN (normotensive animals). This facilitation seems to be qualitatively and quantitatively similar in male and female SH rats and female NZH rats. That the facilitatory mechanisms are located presynaptically can be inferred from the comparisons of the effects of isoprenaline and angiotensin II on the pressor responses to PNS and infusion of exogenous NA.

The results also suggest that the uptake I mechanism seems more sensitive to cocaine in the kidney than in the mesenteric vasculature. The isolated kidney seemed to have an increased pressor response to PNS and NA infusion compared to the isolated mesenteric vasculature. This could result from the tissue being more sensitive, possibly due to increased number of postsynaptic receptors and/or increased facilitatory mechanisms.

However, it should be noted that the rate of perfusion in the isolated perfused mesenteric vasculature was 5 ml/min and the perfusion rate for the isolated kidney preparation was 10 ml/min.

This increased perfusion rate could affect the basal perfusion

pressure and the difference in the perfusion rates could also, clearly, have affected the pressor responses to PNS and bolus injections of NA.

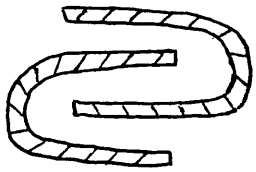
CHAPTER 4

EXPERIMENTAL HYPERTENSION: GOLDBLATT, TWO-KIDNEY, ONE-CLIP HYPERTENSION

4.1. METHODS

4.1i Goldblatt, two-kidney, one-clip hypertension

Silver clips were made from silver tape by bending double a 5 mm x 2 mm x 0.2 mm thick piece of the tape over another to give a clip with an internal diameter of 0.2 mm (silver tape supplied by Johnson Mathey & Co. Ltd).
Fig 4.1.



Male and female Wistar rats, aged four to five weeks, were anaesthetised with a mixture of Hypnorm (each millilitre containing 0.315 mg fentanyl citrate and 10 mg fluanisone), water and Hypnovel (each millilitre containing 5 mg of midazolam) in the proportion,

Hypnorm: Water for injection : Hypnovel (midazolam 5 mg/ml)

1 volume : 2 volumes : 1 volume

An intraperitoneal injection of 0.3 ml/100g body weight of the above mixture was used.

A small patch of fur was shaved off on the side of the animal above the right kidney. A small incision was made

to expose the kidney and the major renal blood vessels. The renal artery was gently separated from the renal vein and a silver clip (internal diameter, 0.2 mm) was put around the renal artery in order to occlude the artery. The animals were then sutured up with Michel clips and left in a warm cage to gain consciousness, which usually occurred twenty to thirty minutes after the surgery.

In the sham operated animals a small incision on the side of the animal above the right kidney was made and the kidney exposed. The incision was then sutured up using Michel clips and the animals were transferred to a warm cage to regain consciousness.

Once the animals had regained consciousness they were left in the cages for one to two weeks before being handled.

For clarity,

Goldblatt, two kidney-one clip rats are referred to as renal hypertensive rats, W_mR and W_fR refer to male and female animals respectively.

Sham operated animals are referred to as control Wistar rats, W_m and W_f refer to male and female animals respectively.

4.1ii Blood pressure measurements

Systolic blood pressure was monitored in the rats when the animals had recovered from the operation, usually one to two weeks after the surgery (i.e. seven weeks old). Systolic blood pressure was monitored in the conscious animals by the tail-cuff method using a programmed electro-sphygmomanometer PE-300 (Narco bio-instruments) coupled to a flat-bed recorder (CR-6505, J.J. instruments). The systolic blood pressure was measured weekly; the average of three measurements was taken as the mean systolic blood pressure.

4.1iii Chronic ICI 118,551 treatment

At the age of seven weeks, some of the male and female renal hypertensive rats were randomly chosen for chronic treatment with ICI 118,551 and set aside in separate cages.

Chronic treatment with ICI 118,551 was started when the animals were eight weeks old and was carried on until they were between twelve and thirteen weeks old, when they were sacrificed. Chronic treatment with ICI 118,551 consisted of an oral daily dose of ICI 118,551, 25 mg/kg rat body weight.

For clarity,

Goldblatt, two-kidney, one-clip rats chronically treated with ICI 118,551 are referred to as renal hypertensive rats chronically treated with ICI 118,551, W_mRI and W_fRI refer to male and female animals respectively.

4.1iv Isolated mesenteric vascular bed preparation

The animals were sacrificed at twelve weeks of age and the isolated perfused mesenteric vasculature was set up as previously described in Section 2.2i. The preparation was placed in a 5 ml water-jacketed organ bath maintained at 37°C and perfused with a modified Kreb's solution (composition as described in Section 2.2i) at a constant flow rate of 5 ml/min by means of a Watson Marlow peristaltic pump.

The perfusing solution was aerated with a mixture of 95% oxygen and 5% carbon dioxide before passing through a warming coil maintained at 38°C. Changes in perfusion pressure were measured at a point close to the cannula by means of a pressure transducer (Bell and Howell, type 4-422-0001) and recorded on a Devices (M2) recorder.

After allowing time for the basal perfusion pressure to stabilize, usually 15 minutes, the isolated perfused mesenteric vasculature was subjected to either PNS or to

a bolus of NA. PNS was delivered at 5 minute intervals via bipolar platinum electrodes placed around the superior mesenteric artery. Supramaximal rectangular pulses, 1 msec, 80V were applied for 10 sec. at 30Hz by means of a Grass, model S44, stimulator. The neural basis of the pressor response mediated by stimulation of the arterial adrenergic nerve was confirmed in the preparations from all the groups by abolition of the response after perfusion with guanethidine (0.01mM, n=3 animals for each group). NA was injected directly into the perfusate proximal to the arterial cannula at 5 minute intervals in a volume of 0.1 ml. of a 10^{-6}M solution.

Once stable responses to PNS and NA infusion had been demonstrated, perfusion with other drugs began. The method of perfusion of the drugs was as described previously in Section 2.2i.

Data was derived from the second response to PNS or NA infusion; the exception being experiments involving cocaine, when the first response was taken.

PNS and NA response data after drug perfusion in the preparations are expressed as a percentage of the control pressor response in order to standardize the data.

Age matched male and female Wistar rats (University of Bath strain) were used in this study. The animals used were supplied by the University of Bath Animal House.

4.1v Statistical Analysis

Results were analysed using Student's t-test for group and paired mean comparisons. Probability levels equal to or less than 0.05 were taken as indicating statistically significant differences. All comparisons employed the two-tailed test.

4.2 Results

4.2i Progression of the Hypertension in the Goldblatt, two- kidney, one-clip hypertensive rat

The degree of hypertension induced by the two-kidney,one - clip procedure and the effect of chronic treatment with ICI 118,551 (25 mg/kg, p.o., daily) on this hypertension is illustrated in Figs 4.2 and 4.3 for the male and female rats respectively.

Chronic treatment with ICI 118,551 attenuated the degree of hypertension in the renal hypertensive animals though it did not altogether stop the progression of the hypertension.

There were no significant differences observed in the progression of the hypertension, or the effect of chronic treatment with ICI 118,551 on this hypertension, between the males and the females.

Table 4.1. shows the pressor responses to PNS and NA infusion in the isolated mesenteric vasculature of all the groups of rats.

As can be seen from Table 4.1, the pressor responses to PNS and NA infusion were significantly greater in the preparations from the renal hypertensive animals than in the control Wistar rats. An interesting point to note is

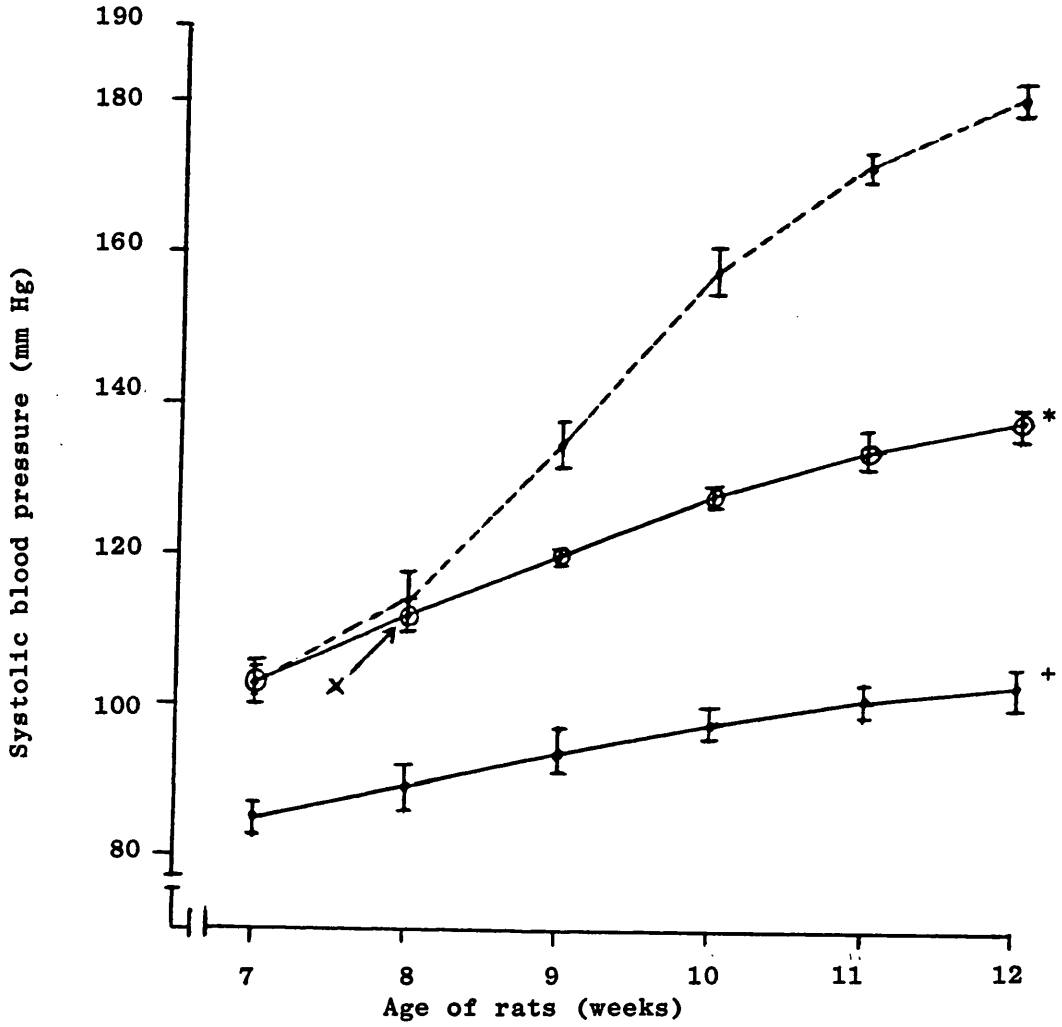


Fig 4.2

Comparison of systolic blood pressure in male rats.
Point "x" indicates age when chronic treatment of two-kidney, one-clip renal hypertensive rats with ICI 118,551 was begun.
Vertical lines indicate s.e. of mean. n = number or rats in each group.
•---•, n=9; two-kidney, one-clip renal hypertensive rats
•——•, n=4; sham-operated rats
⊙——⊙, n=6; renal hypertensive rats treated chronically with ICI 118,551
+ $p<0.05$, significantly different from the other two groups
* $p<0.05$, significantly different from untreated renal hypertensive rats from age 9 weeks.

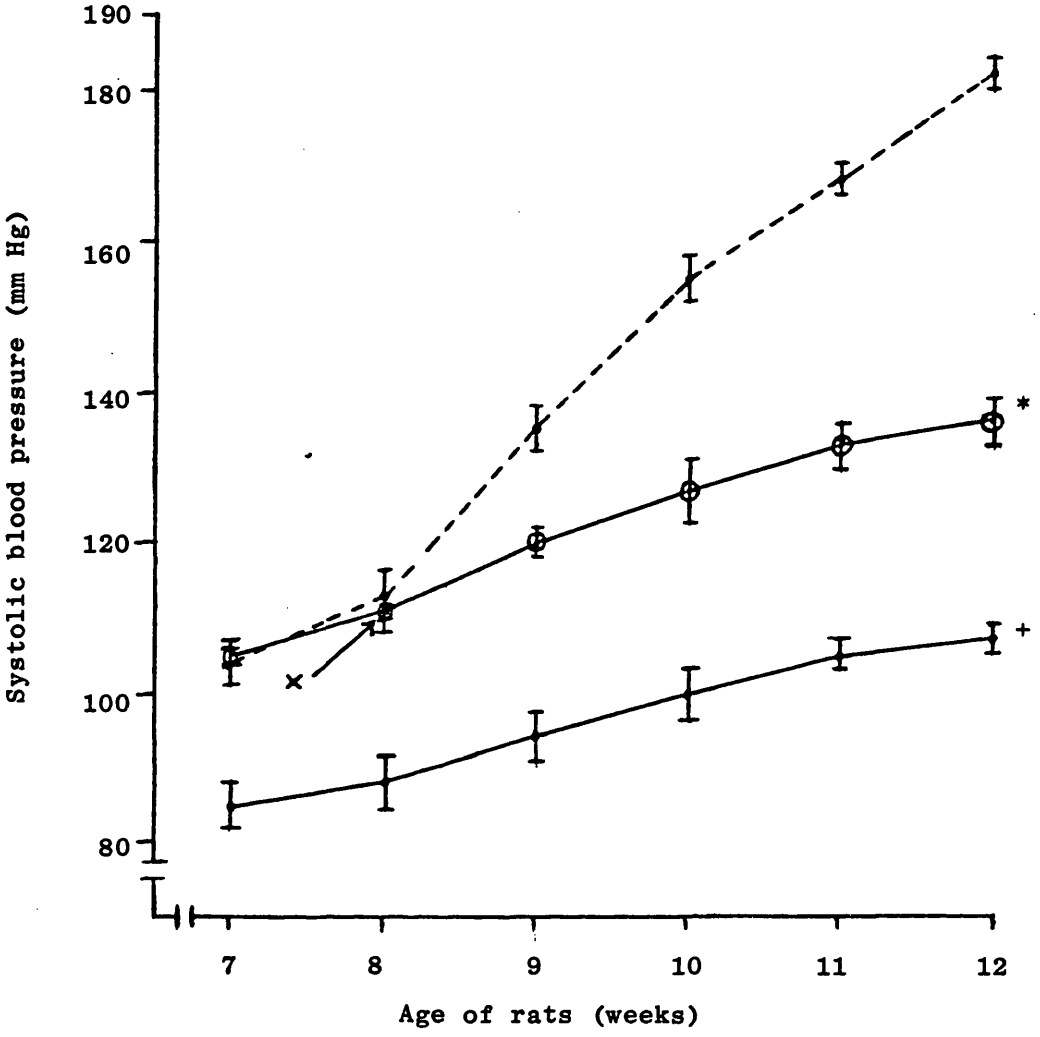


Fig 4.3

Comparison of systolic blood pressure in female rats.
Point "x" indicates age when chronic treatment of two-kidney, one-clip renal hypertensive rats with ICI 118,551 was begun.
Vertical lines indicate s.e. of mean. n = number of rats in each group.
•---•, n=9; two-kidney, one-clip renal hypertensive rats
•——•, n=4; sham-operated rats .
⊙——⊙, n=6; two-kidney, one-clip renal hypertensive rats treated chronically with ICI 118,551.
+ $p<0.05$, significantly different to other two groups.
* $p<0.05$, significantly different from untreated renal hypertensive rats from age 9 weeks.

Table 4.1

Pressor responses to PNS and NA infusion in the isolated mesenteric vasculatures of male and female renal hypertensive rats, male and female renal hypertensive rats treated chronically with ICI 118,551 and sham-operated male & female Wistar rats

	Pressor response Δ mm Hg	
	PNS (30Hz,80v,10sec)	NA infusion (10^{-6} M solution)
Male renal hypertensive	+ 9.5 ± 0.5 (21)	12.5 ± 0.5 (12)
Male renal hypertensive + ICI 118,551	8.2 ± 0.2 (12)	12.6 ± 1.1 (6)
Male Wistar rat, sham operated	. 6.9 ± 0.4 (20)	. 9.5 ± 0.5 (10)
Female renal hypertensive	+ 9.8 ± 0.2 (21)	12.5 ± 0.5 (12)
Female renal hypertensive + ICI 118,551	8.5 ± 0.3 (12)	13.5 ± 0.7 (6)
Female Wistar rat, sham operated	. 6.5 ± 0.7 (20)	.10.3 ± 0.5 (10)

Values given are mean ± s.e. of mean
Number in parenthesis, number of observations
+ $p<0.05$; significant differences between renal hypertensive rats and all other groups.
. $p<0.05$; significant differences between sham operated animals and all other groups.

that the pressor response to PNS in the preparation from the renal hypertensive rat was also greater than that from the animals chronically treated with ICI 118,551.

Basal perfusion pressure was slightly, but significantly elevated in the preparations from the renal hypertensive animals. Table 4.2.

4.2ii Effect of isoprenaline on the pressor responses to PNS and NA infusion in the preparations from renal hypertensive rats, renal hypertensive rats treated chronically with ICI 118,551 and Wistar rats

At the concentrations employed in this study (10^{-9}M to 10^{-6}M), isoprenaline alone had no significant effect on the basal perfusion pressure of the isolated mesenteric vascular preparation from any of the groups. In the preparations from the untreated renal hypertensive and control animals, isoprenaline caused a dose dependent potentiation of the pressor response to PNS at the lower isoprenaline concentrations (10^{-9}M to $5x10^{-8}$M). However, the degree of the isoprenaline-induced potentiation of the pressor response to PNS in the untreated renal hypertensive animals was significantly greater than that in the control animals at all concentrations of isoprenaline except for $5x10^{-8}$M in the female preparations. At higher isoprenaline concentrations ($>5x10^{-8}$M) inhibition of the vasoconstrictor response to PNS was observed in the normotensive Wistar rats.

Table 4.2

Basal perfusion pressure of isolated perfused mesenteric vasculatures of male and female renal hypertensive rats, male and female renal hypertensive rats treated chronically with ICI 118,551 and sham operated Wistar rats.

	Basal perfusion pressure (mm Hg)
Male renal hypertensive	41.5 ± 2.3 (10)
Male renal hypertensive + ICI 118,551	40.5 ± 2.5 (10)
Male Wistar rat, sham operated	. 33.3 ± 2.9 (10)
Female renal hypertensive	40.3 ± 1.3 (10)
Female renal hypertensive + ICI 118,551	39.6 ± 0.6 (10)
Female Wistar rat, sham operated	. 31.6 ± 3.4 (10)

Values are mean ± s.e. of mean
Number in parenthesis, number of animals
. $p<0.05$; significant difference between sham operated animals and all other groups

Isoprenaline did not have any effect on the pressor response to PNS in the renal hypertensive animals treated chronically with ICI 118,551. Fig 4.4 a,b for preparations from male and female rats respectively.

As can be seen from Fig 4.5 a,b, for preparations from male and female rats respectively, isoprenaline caused a dose dependent inhibition of the pressor response to NA infusion in the preparations from untreated renal hypertensive animals, renal hypertensive animals chronically treated with ICI 118,551 and normotensive Wistar rats.

There was no significant difference in the degree of isoprenaline-induced inhibition of the pressor response to NA infusion in any of the preparations.

In order to investigate the effects of β-adrenoreceptor antagonists, a standard concentration of 10^{-8}M isoprenaline was chosen.

4.2iii Effect of atenolol on the isoprenaline-induced effects on the pressor responses to PNS and NA infusion in the preparations from untreated renal hypertensive rats and Wistar rats

Atenolol (10^{-7}M) was employed as the selective β_1-adrenoreceptor antagonist. At this concentration atenolol had no significant effect on either the basal perfusion

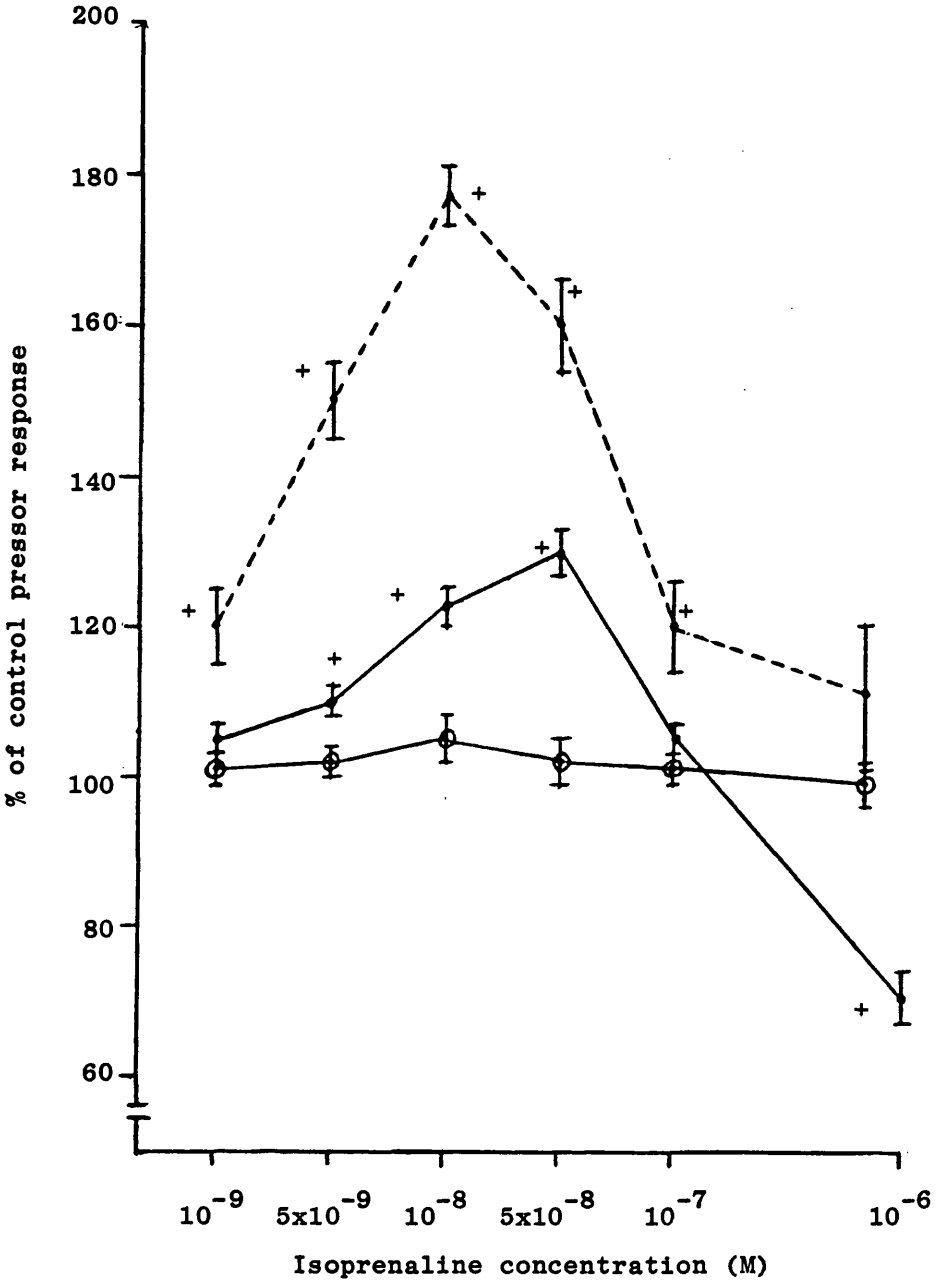


Fig 4.4a

Effect of isoprenaline on the pressor response to PNS in the isolated perfused mesenteric vasculatures of two-kidney, one-clip renal hypertensive male rats (WmR, .---., n=3), sham-operated male rats (Wm, •——•, n=4) and the renal hypertensive male rats chronically treated with ICI 118,551 (WmRI, ⊙——⊙, n=3) Vertical lines indicate s.e. of mean. n = number of animals in each group.
WmR compared to Wm $p<0.05$
+ $p<0.05$, compared to WmRI and control pressor response

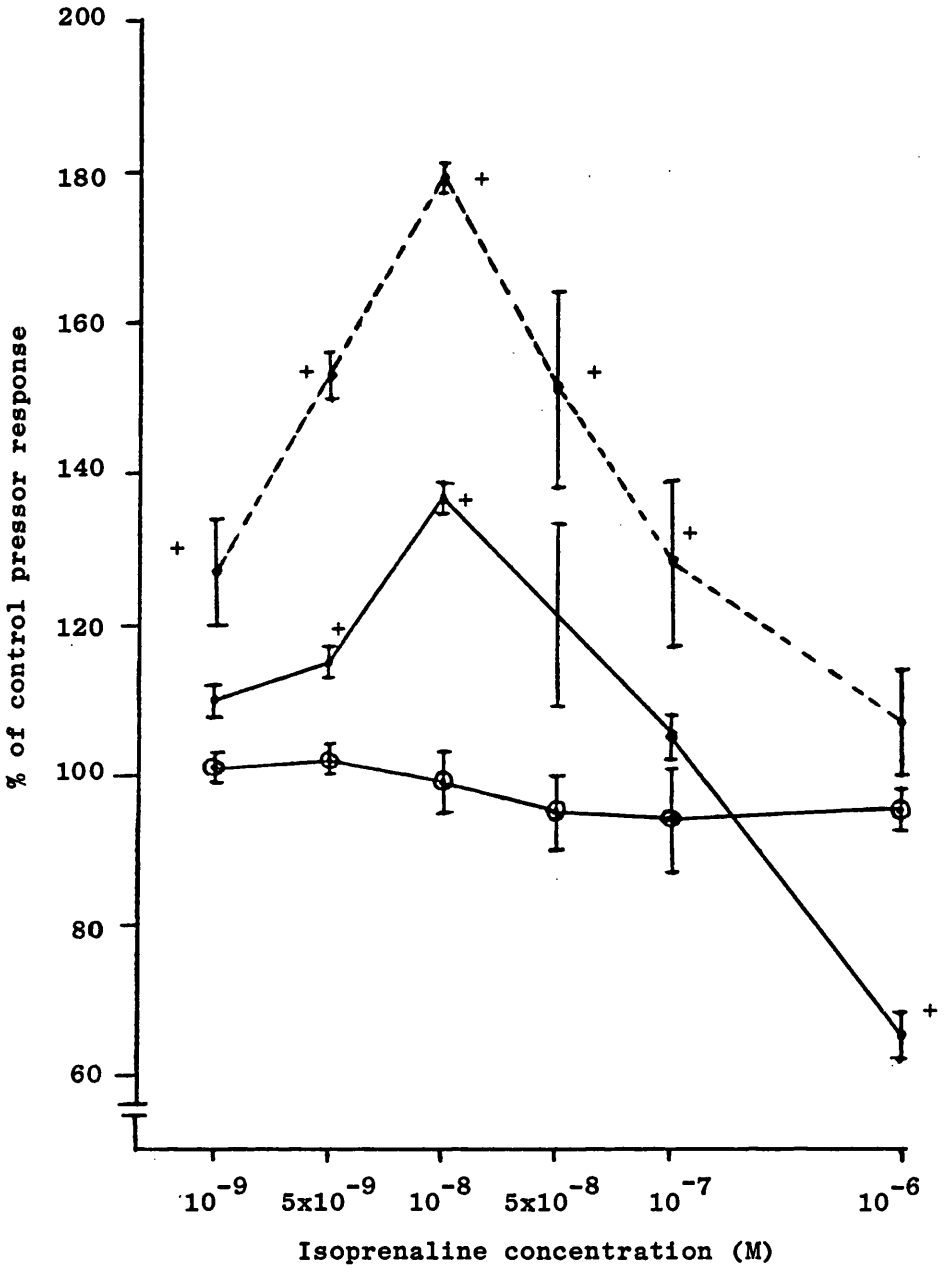


Fig 4.4b

Effect of isoprenaline on the pressor response to PNS in the isolated perfused mesenteric vasculatures of two-kidney, one-clip renal hypertensive female rats (WfR, .---., n=3) sham-operated female rats (Wf, •——•, n=4) and the renal hypertensive female rats chronically treated with ICI 118,551 (WfRI, ⊙—⊙, n=3).
Vertical lines indicate s.e. of mean. n = number of animals in each group.
WfR compared to Wf, $p<0.05$ for all isoprenaline concentrations except 5×10^{-8} M
+ $p<0.05$ compared to WmRI and control response.

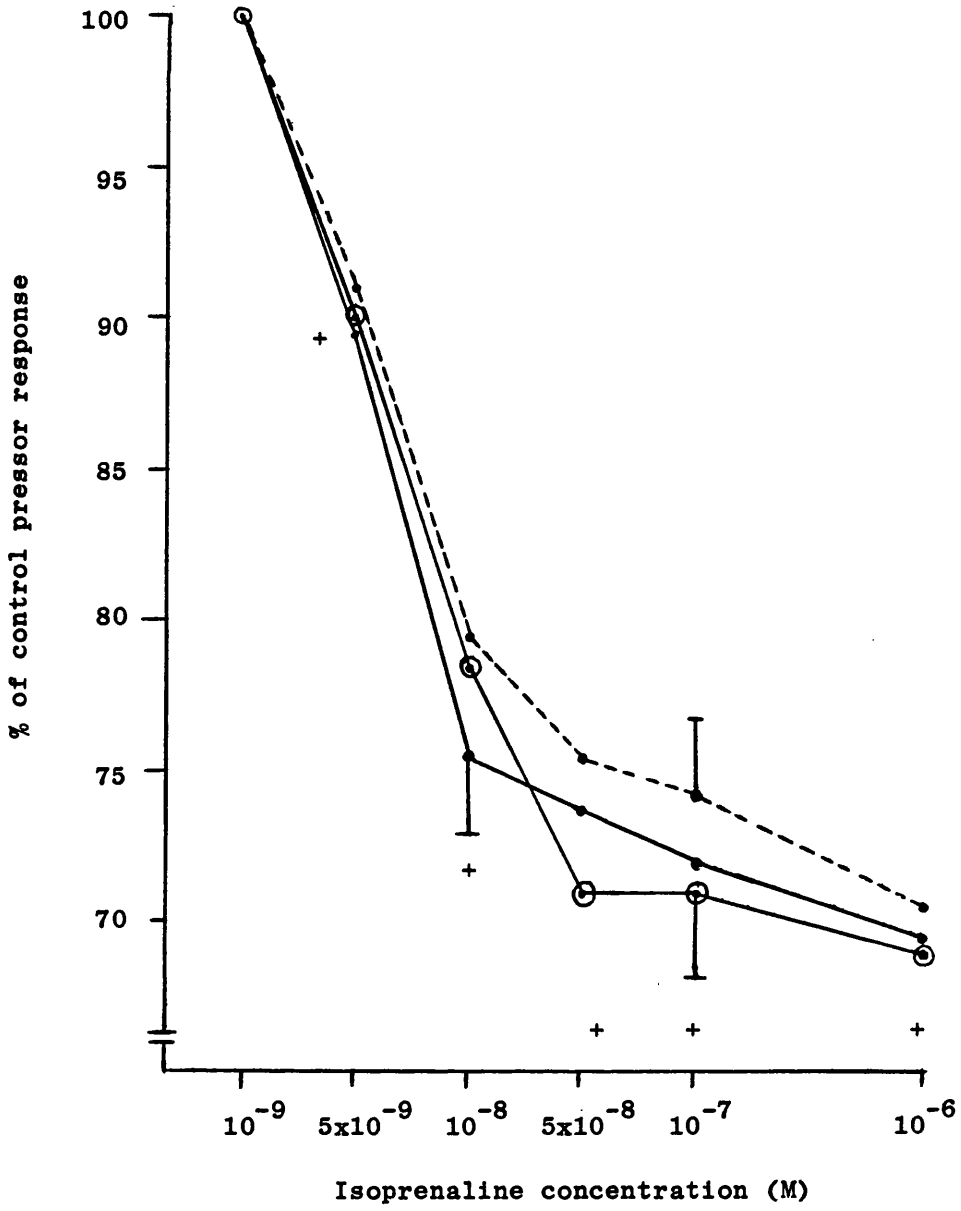


Fig 4.5a

Effect of isoprenaline on the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of two-kidney, one-clip renal hypertensive male rats (.---., n=3), sham-operated male rats (•——•, n=4) and the renal hypertensive male rats treated chronically with ICI 118,551 (⊙——⊙, n=3)
Vertical lines indicate s.e. of mean (most omitted for clarity)
n = number of animals for each group.
+ $p < 0.05$, all three groups compared to control pressor response

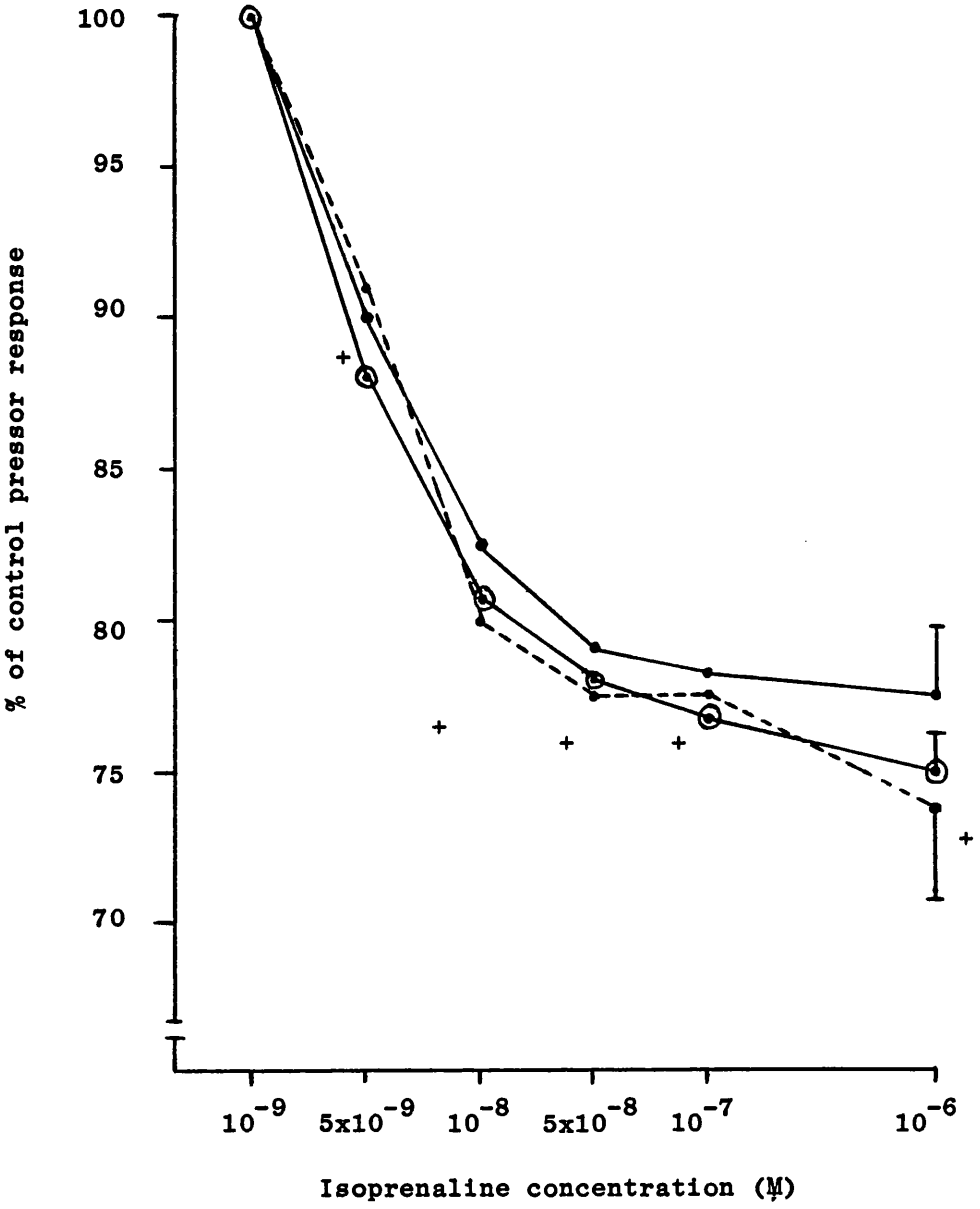


Fig 4.5b

Effect of isoprenaline on the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of two-kidneys, one-clip renal hypertensive female rats (.---., n=3), sham-operated female rats (●——●, n=4) and the renal hypertensive female rats treated chronically with ICI 118,551 (⊙——⊙, n=3)
Vertical lines indicate s.e. of mean (most omitted for clarity)
n = number of animals for each group
+ $p < 0.05$, all three groups compared to control pressor response

pressure or the pressor response to PNS in any of the preparations. Atenolol did not inhibit the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations. Fig 4.6

Atenolol however significantly reversed the isoprenaline induced inhibition of the pressor response to NA infusion in all the preparations. Fig 4.7

4.2iv Effect of ICI 118,551 on the isoprenaline-induced effects on the pressor responses to PNS and NA infusion in the preparations from untreated renal hypertensive rats and Wistar rats

ICI 118,551 ($5x10^{-7}M$) was used as the selective β_2-adrenoreceptor antagonist. At this concentration the antagonist had no significant effect on either the basal perfusion pressure or the pressor responses to PNS and NA infusions in any of the preparations. However, ICI 118,551 completely inhibited the isoprenaline-induced potentiation of the PNS pressor response in preparations from both, the untreated renal hypertensive and normotensive Wistar rats. Fig 4.8.

ICI 118,551 significantly reversed the isoprenaline induced inhibition of the pressor response to NA infusion in the preparations from both types of animals. Fig 4.9.

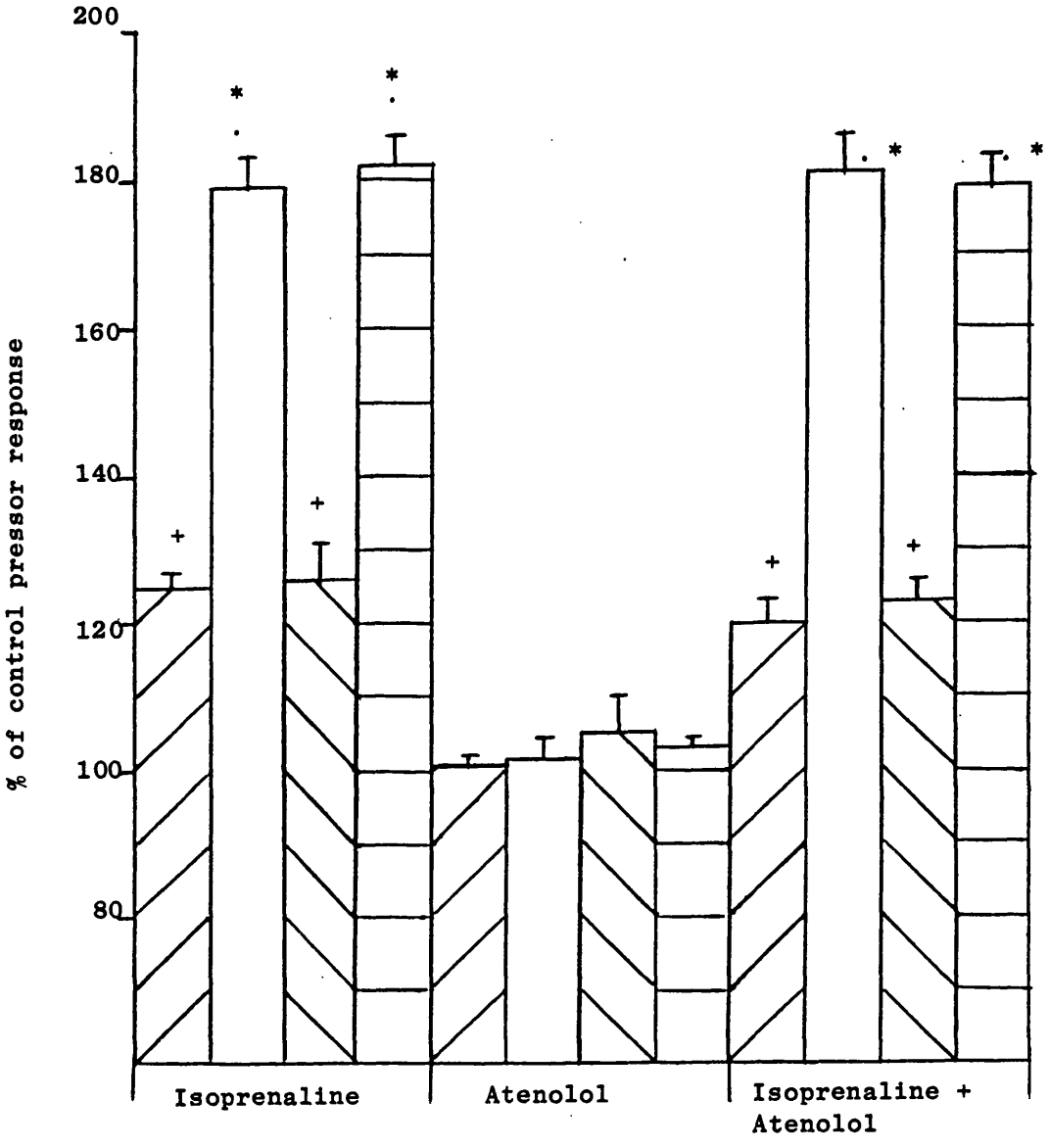


Fig 4.6

Effect of atenolol on the isoprenaline-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR and W_fR respectively) and male and female sham operated rats (W_m and W_f, respectively).
Vertical lines indicate s.e. of mean n = 3 animals for all groups

W_mR W_m

W_fR W_f

+ $p<0.05$ compared to control pressor response
. $p<0.01$ compared to control pressor response
* $p<0.05$ compared to sham-operated rats

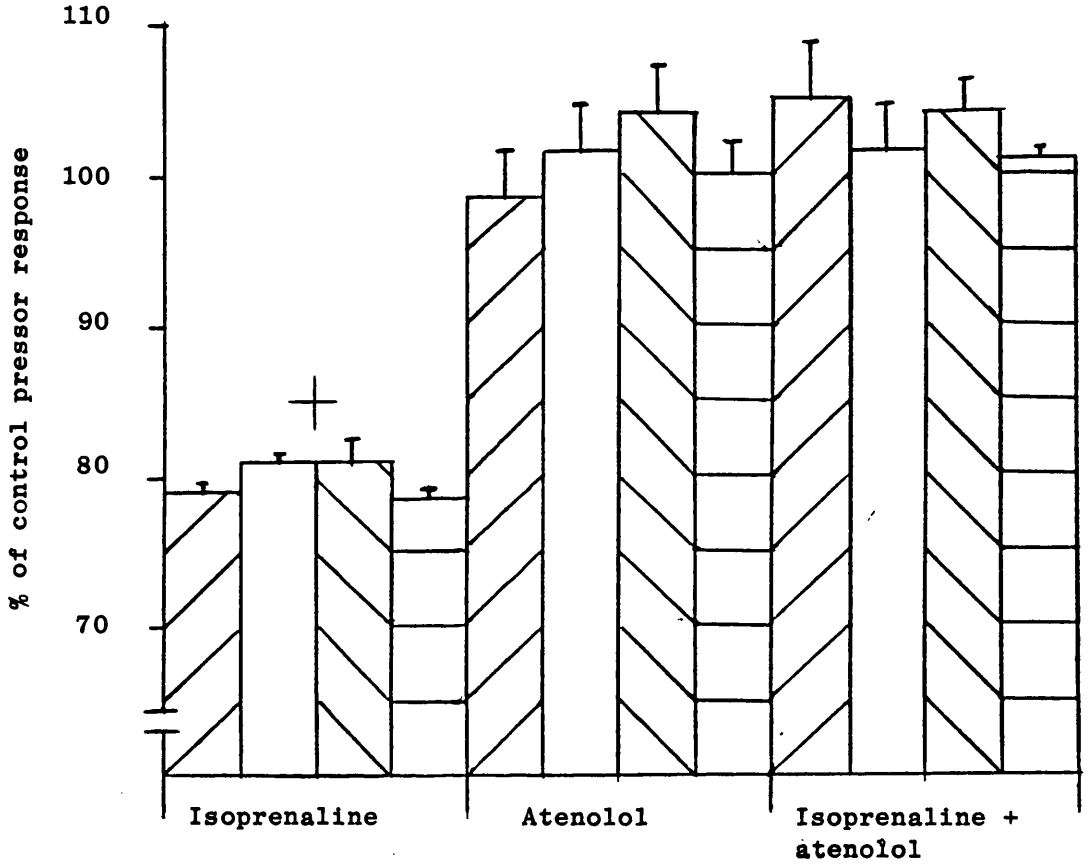


Fig 4.7

Effect of atenolol on the isoprenaline induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR and W_fR, respectively) and male and female sham-operated rats (W_m and W_f, respectively). Vertical lines indicate s.e. of mean. n = 3 animals for each group.

W_mR □ W_m ▨

W_fR ▤ W_f ▧

┼ $p<0.05$, all groups in this section significantly different compared to those in all other sections.

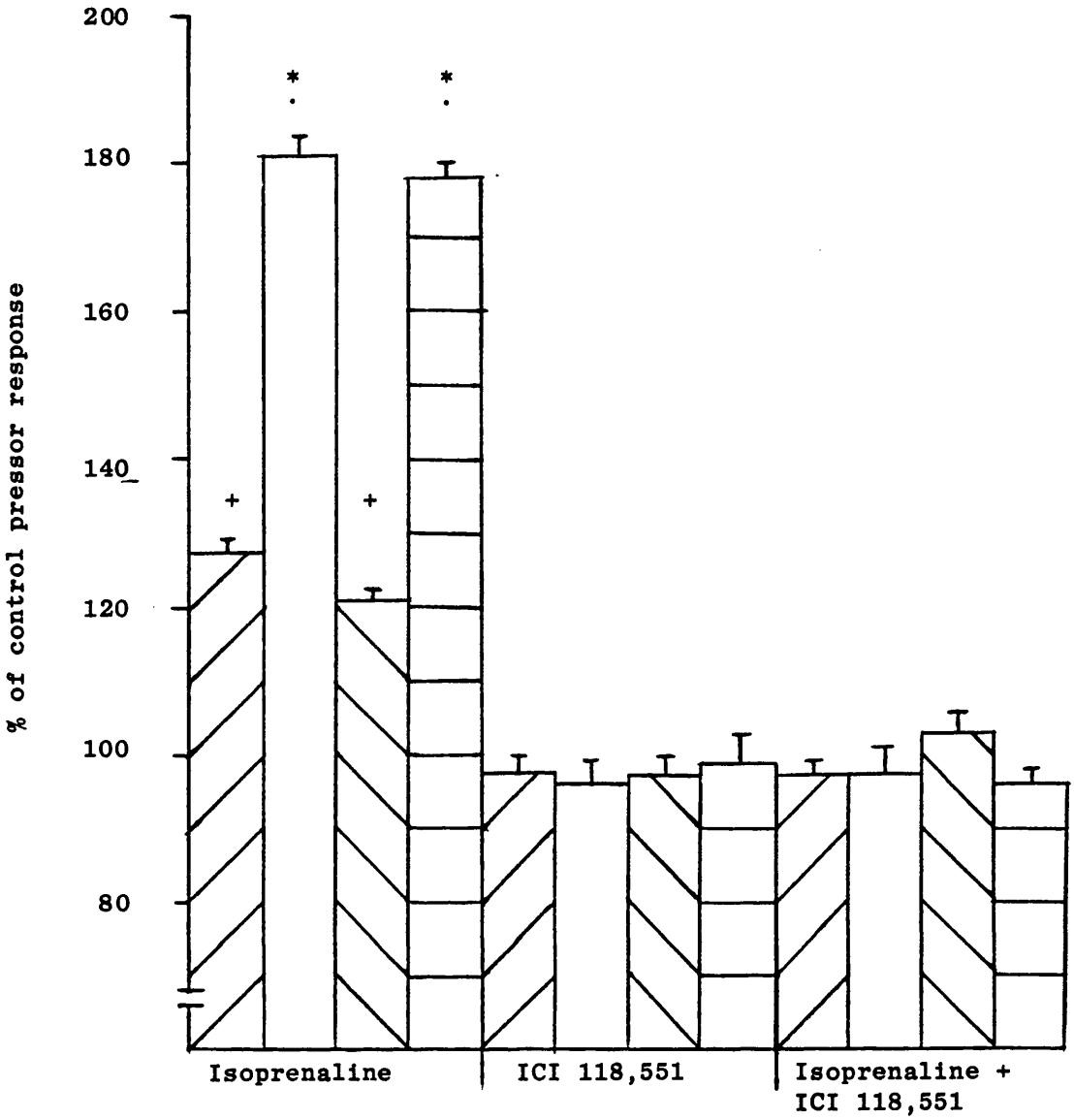


Fig 4.8

Effect of ICI 118,551 on the isoprenaline induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR and W_fR, respectively) and male and female sham-operated rats (W_m and W_f, respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for all groups.

W_mR W_m

W_fR W_f

+ $p<0.05$; significantly different compared to control pressor response

. $p<0.01$; significantly different compared to control pressor response

* $p<0.05$; significantly different compared to sham-operated rats

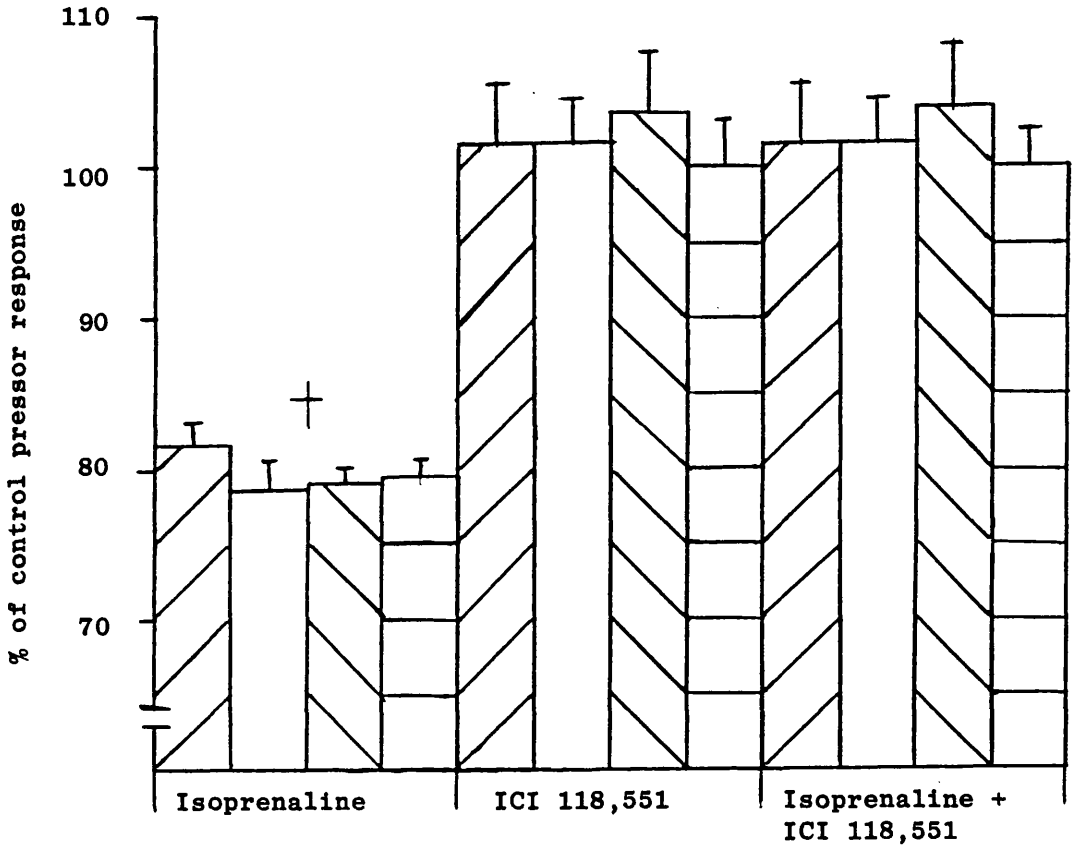


Fig 4.9

Effect of ICI 118,551 on the isoprenaline-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR, respectively) and male and female sham-operated rats (W_m, W_f, respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

W_mR W_m

W_fR W_f

+ $p<0.05$; all groups in this section significantly different to those in all other sections.

4.2v Effect of ICI 118,551 and atenolol on the isoprenaline induced inhibition of the pressor response to NA infusion in the renal hypertensive rats treated chronically with ICI 118,551

Fig 4.10 illustrates the effect of ICI 118,551 and atenolol on the isoprenaline induced inhibition of the pressor response to NA infusion in preparations from renal hypertensive animals chronically treated with ICI 118,551. Atenolol and ICI 118,551 significantly reversed the isoprenaline induced inhibition of the NA pressor response.

4.2vi Effect of angiotensin II (AII) and cocaine on the pressor response to PNS and NA infusion in the preparations from untreated rats, renal hypertensive rats, renal hypertensive rats treated chronically with ICI 118,551 and normotensive Wistar rats

Angiotensin II (10 ng/ml) had no significant effect on the basal perfusion pressure of any of the preparations. Angiotensin II significantly potentiated the pressor response to PNS in all the preparations. The degree of this potentiation was greatest in the preparations from the untreated renal hypertensive animal followed by preparations from the ICI 118,551 treated animals and control animals. Preparations from renal hypertensive animals treated chronically with ICI 118,551 and untreated

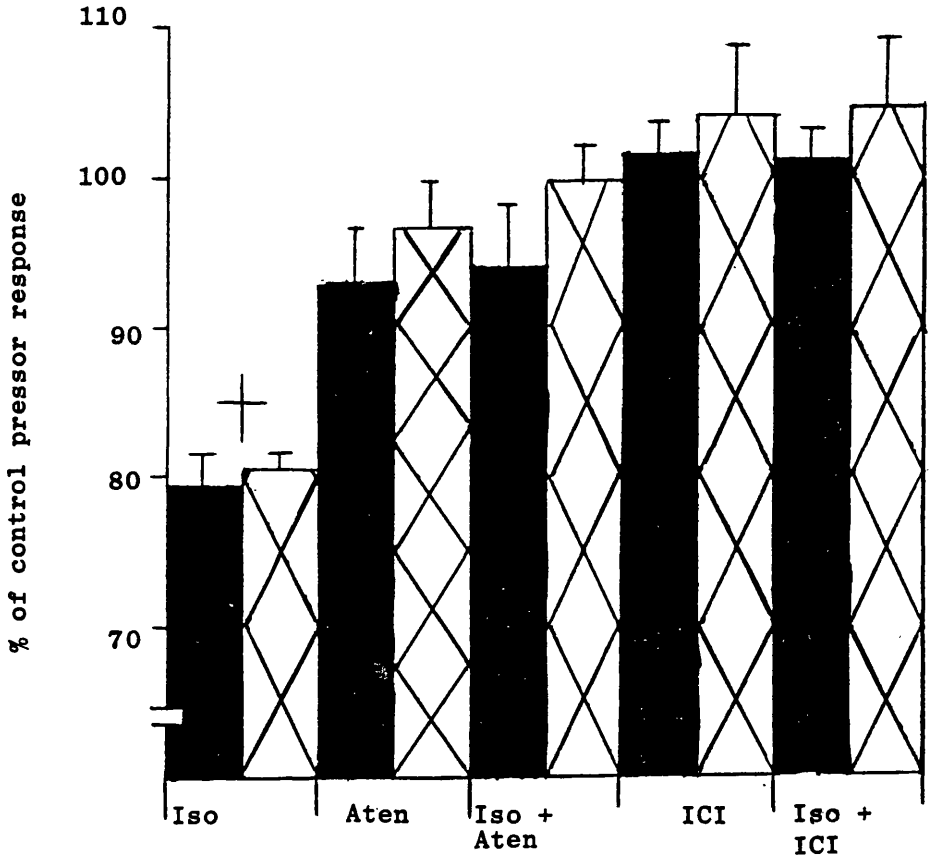


Fig 4.10

Effect of atenolol (aten) and ICI 118,551 (ICI) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats chronically treated with ICI 118,551 (W_mRI, W_fRI, respectively). Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mRI ■ W_fRI ⊠

┼ $p<0.05$; all groups in this section significantly different to those in all other sections.

renal hypertensive animals exhibited a significantly greater angiotensin II-induced potentiation of the pressor response to PNS than that from the control normotensive Wistars. Fig 4.11.

The degree of the angiotensin II induced potentiation of the pressor response to NA infusion in the preparations from the renal hypertensive rats treated chronically with ICI 118,551 and untreated renal hypertensive animals was significantly greater ($p<0.05$) than that in the preparations from the control Wistar animals. Fig 4.12.

Only in the preparations from the renal hypertensive animals treated chronically with ICI 118,551 and untreated renal hypertensive animals was the degree of angiotensin II-induced potentiation of the PNS pressor response significantly greater than that of the NA infusion ($p<0.05$).

The specific angiotensin II receptor antagonist, Sar. (200 ng/ml), which alone did not significantly affect the basal perfusion pressure or the pressor response to PNS and NA infusion, completely abolished the angiotensin II-induced potentiation of the pressor response to PNS and NA infusion in all the preparations. Figs 4.11 and 4.12.

The effects of cocaine (5 ug/ml), which alone had no significant effect on the basal perfusion pressure, on the pressor response to PNS and NA infusion are illustrated in

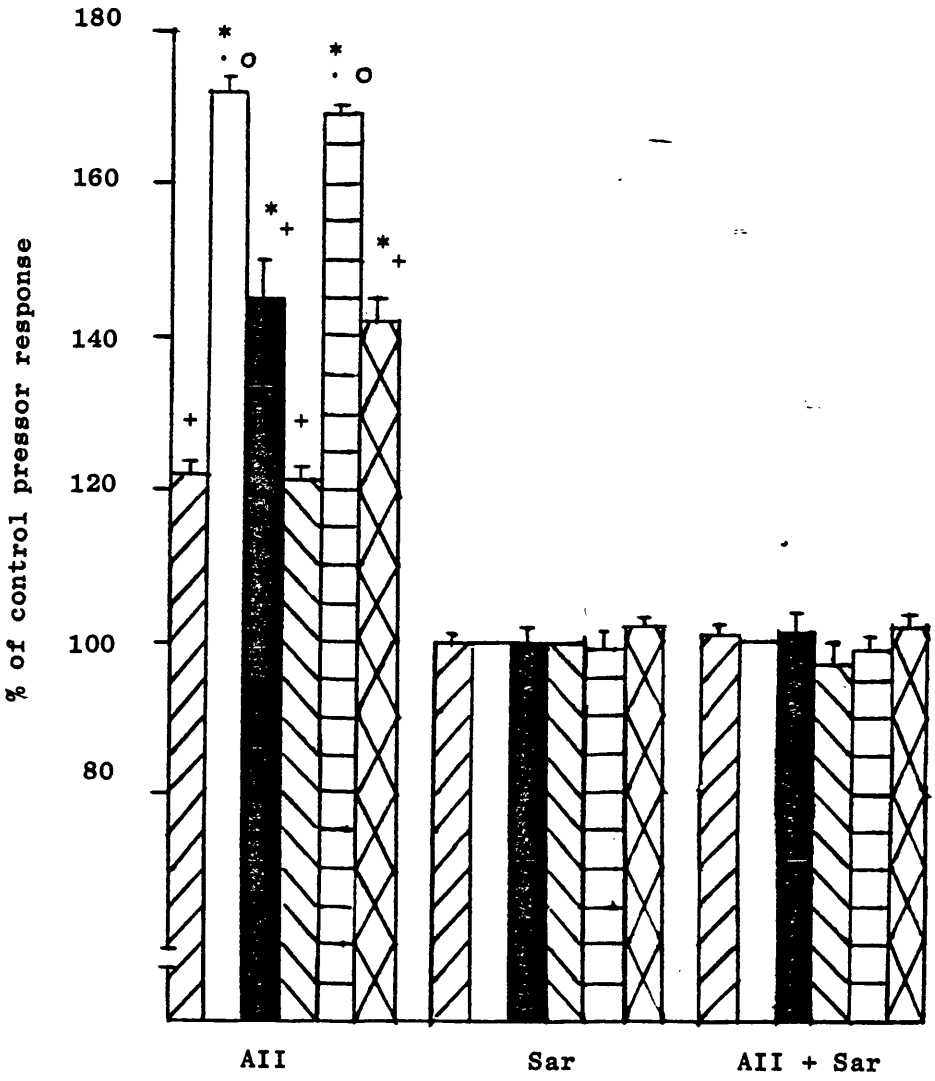


Fig 4.11

Effect angiotensin II (AII) and [Sar^1-Ile^8] angiotensin II (Sar) on the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham operated rats (W_m, W_f respectively) and male and female renal hypertensive rats chronically treated with ICI 118,551 (W_mRI, W_fRI respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m W_mRI

W_fR W_f W_fRI

+ $p<0.05$ compared to control pressor response
. $p<0.01$ compared to control pressor response
* $p<0.05$ compared to W_m & W_f
o $p<0.05$ compared to W_mRI & W_fRI

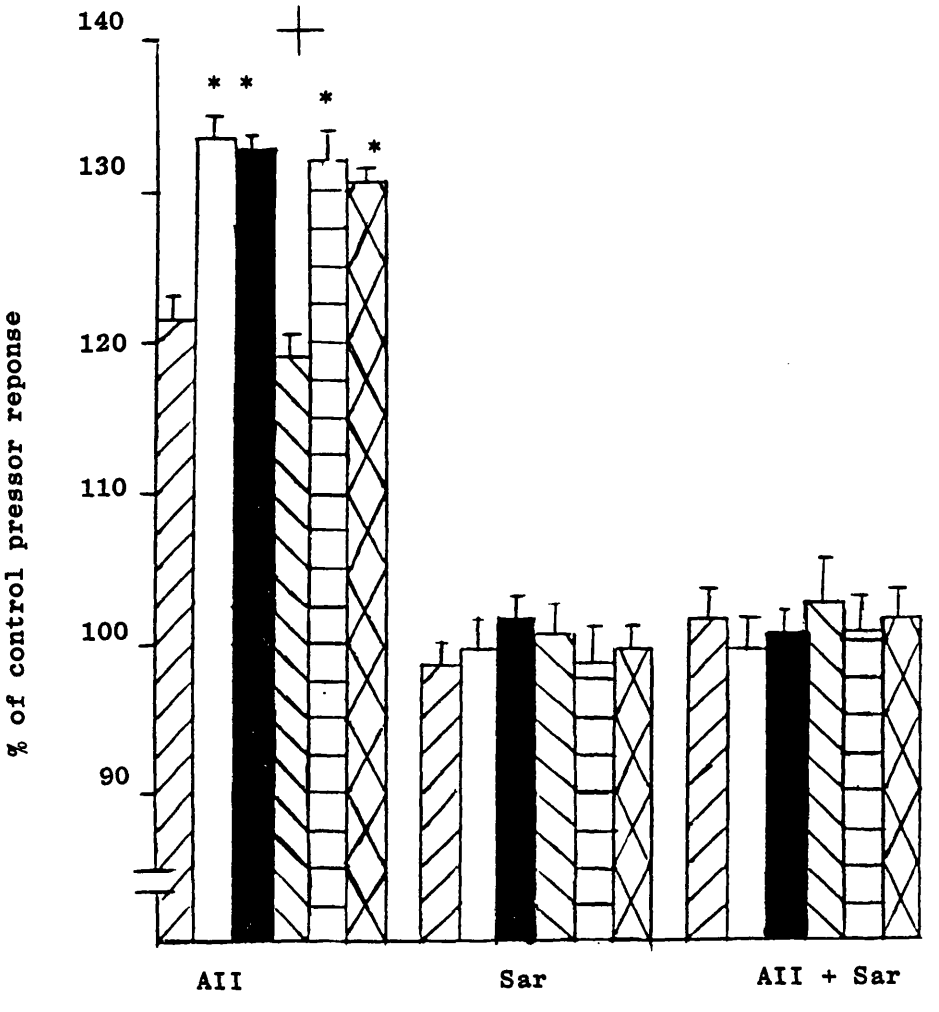


Fig 4.12

Effect of angiotensin II (AII) and Sar^1-Ile^8 angiotensin II (Sar) on the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham-operated rats (W_m, W_f respectively) and male and female renal hypertensive rats chronically treated with ICI 118,551 (W_mRI, W_fRI respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m W_mRI

W_fR W_f W_fRI

* $p<0.05$ compared to sham-operated rats
+ $p<0.05$, groups in this section significantly different to control pressor response

Figs 4.13 and 4.14. Cocaine significantly potentiated the pressor response to PNS and produced a moderate potentiation of the NA infusion pressor response in all the preparations. The degree of potentiation of the PNS pressor response in the preparations from the renal hypertensive rats was greater than that from the renal hypertensive rats treated chronically with ICI 118,551 which in turn was greater than that from the normotensive Wistar rats.

No significant difference in the degree of potentiation of the pressor response to NA infusion between any of the preparations from any of the animals was observed.

When AII (10 ng/ml) was administered together with cocaine (5 µg/ml), the potentiation of the pressor response to both PNS and NA infusion was potentiated further and appeared to be additive.

The facilitatory effect of AII on the PNS pressor response was greatest in the preparations from the renal hypertensive animals followed by that in the renal hypertensive animals treated chronically with ICI 118,551 and least in the preparations from the normotensive animals both in the presence of cocaine, as well as in its absence.

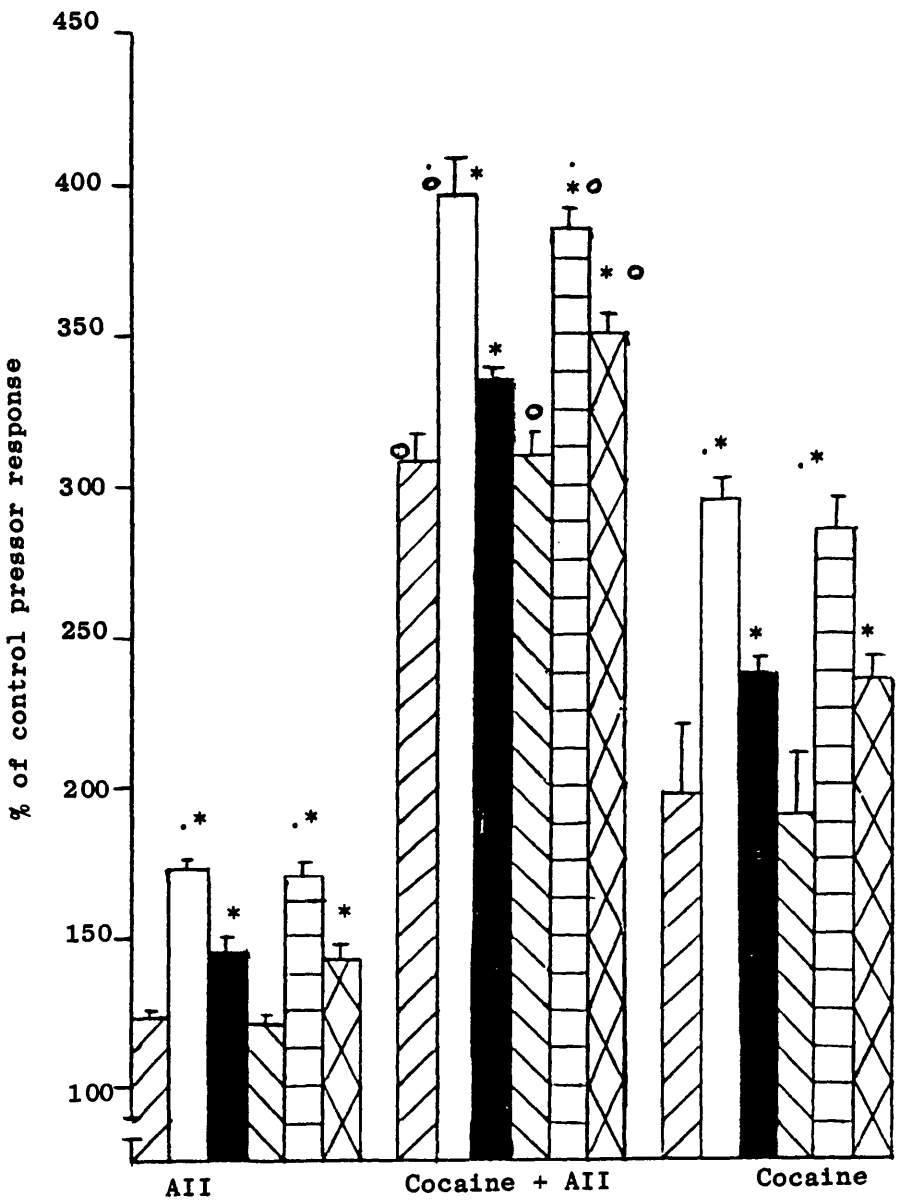


Fig 4.13

Effect of cocaine on the angiotensin II (AII)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham-operated rats (W_m, W_f respectively) and male and female renal hypertensive rats treated chronically with ICI 118,551 (W_mRI, W_fRI respectively)
Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m W_mRI

W_fR W_f W_fRI

* $p < 0.05$ compared to sham-operated rats
. $p < 0.05$ compared to renal hypertensive rats chronically treated with ICI 118,551
O $p < 0.05$ compared to cocaine response section

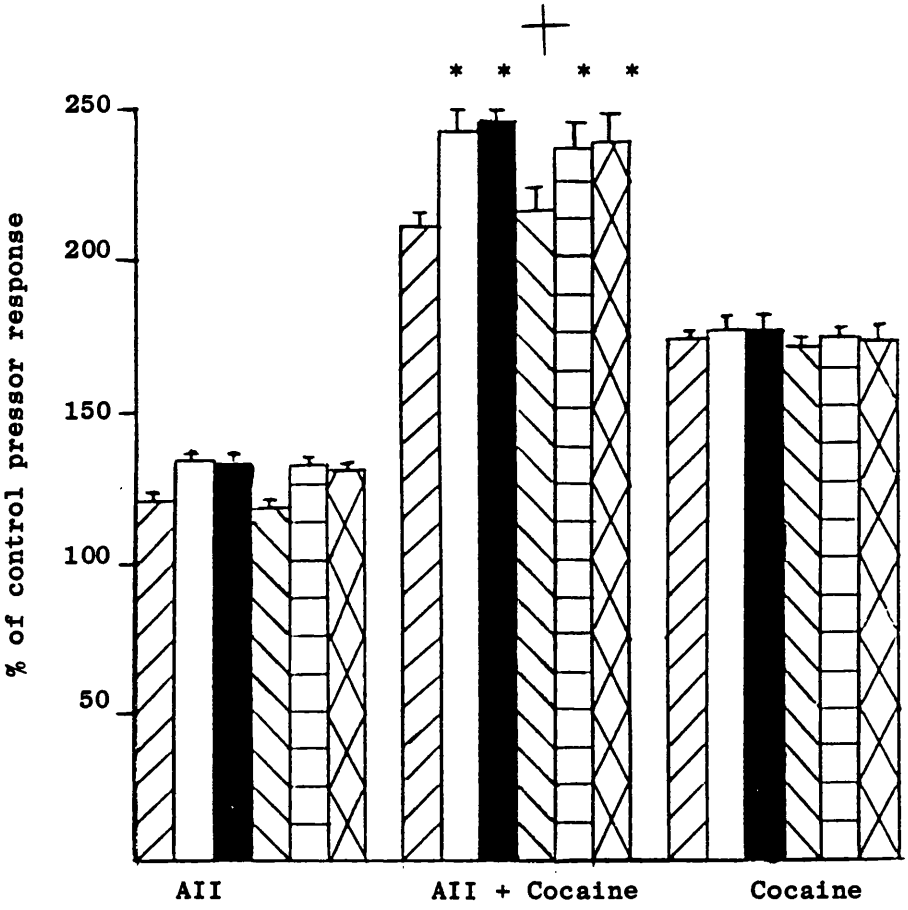
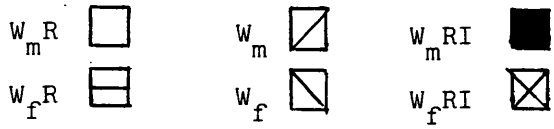


Fig 4.14

Effect of cocaine on the angiotensin II (AII)-induced potentiation of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham-operated rats (W_m, W_f) and male and female renal hypertensive rats treated chronically with ICI 118,551 (W_mRI, W_fRI respectively) Vertical lines indicate s.e. of mean. n = 3 animals for each group

+ $p<0.05$; groups in this section significantly different from those in cocaine section

* $p<0.05$, compared to sham-operated rats



4.2vii Effect of captopril on the isoprenaline induced effects on the pressor response to PNS and NA infusion

Captopril ($5x10^{-6}M$) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. However captopril effectively inhibited the isoprenaline induced potentiation of the pressor response to PNS in the preparations from the untreated renal hypertensive animals and normotensive animals whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion in any of the preparations. Figs 4.15 and 4.16.

Effect of Sar on the isoprenaline induced effects on the pressor response to PNS and NA infusion

Sar (200 ng/ml) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. Sar effectively inhibited the isoprenaline induced potentiation of the PNS pressor response in the preparations from untreated renal hypertensive rats and Wistar rats whilst not having any significant effect on the isoprenaline induced inhibition of the NA infusion pressor response in any of the preparations. Figs 4.17 and 4.18.

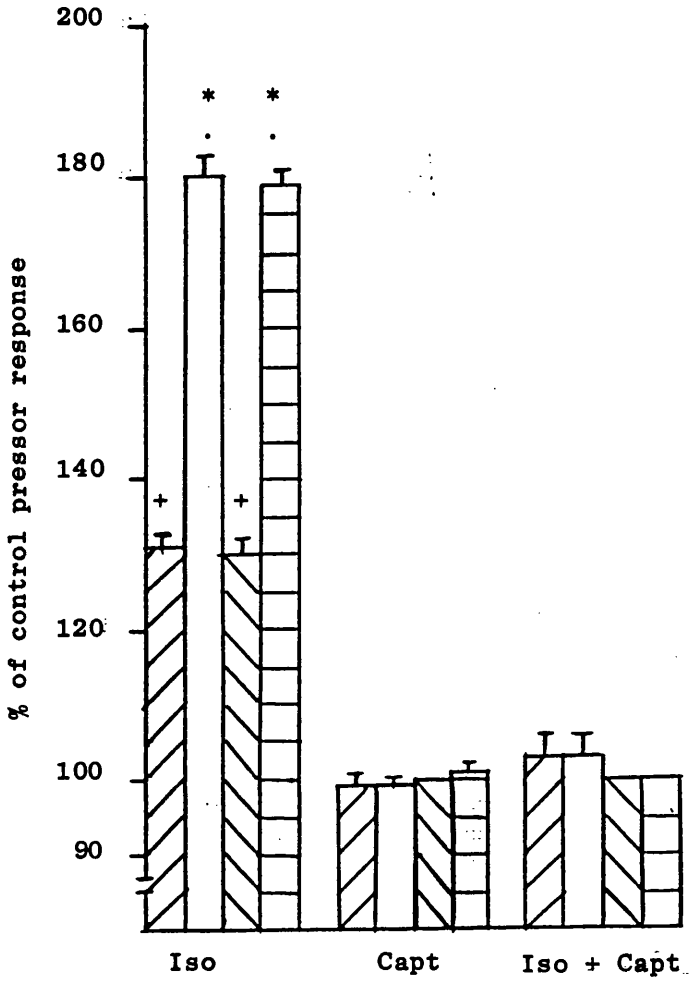


Fig 4.15

Effect of Captopril (Capt) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively and male and female sham-operated rats W_m, W_f respectively)
Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m

W_fR W_f

\+ $p<0.05$, compared to control pressor response
. $p<0.01$, compared to control pressor response
* $p<0.01$, compared to sham-operated rats

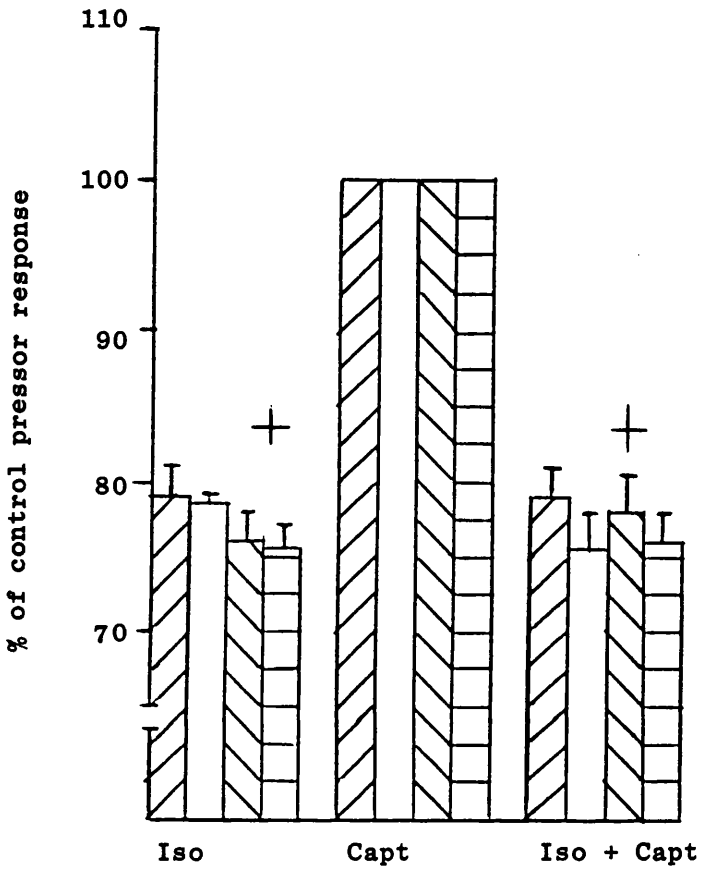


Fig 4.16

Effect of captopril (Capt) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), and male and female sham-operated rats (W_m, W_f respectively). Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m

W_fR W_f

+ $p<0.05$, groups in these sections significantly different to those in Captopril section.

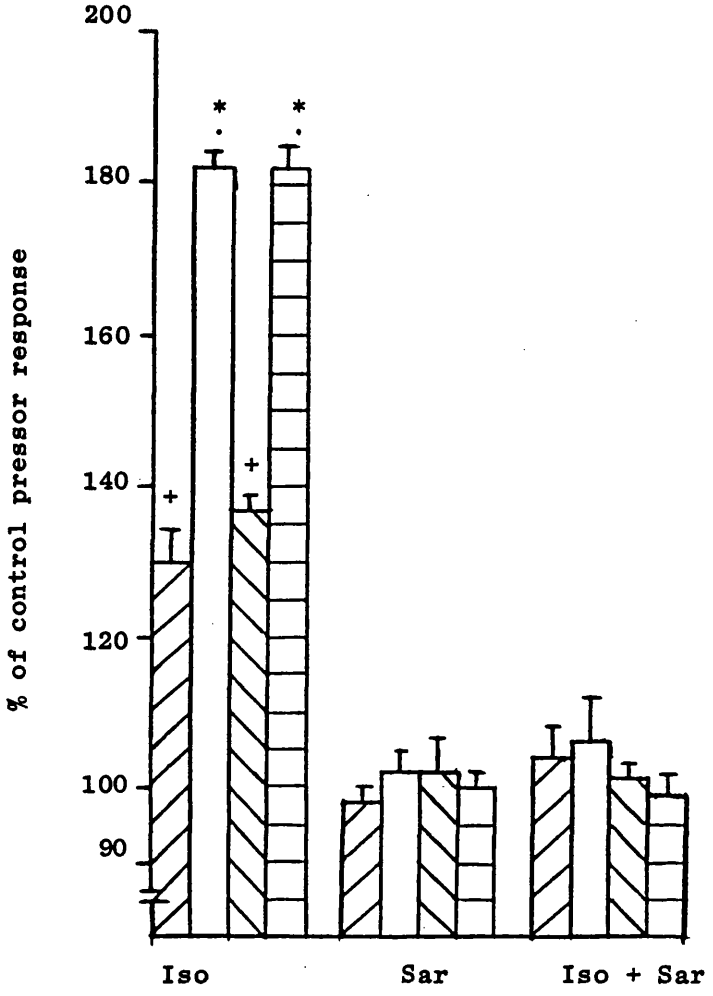


Fig 4.17

Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively) and male and female sham-operated rats (W_m, W_f respectively). Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m

W_fR W_f

+ $p<0.05$, compared to control pressor response
. $p<0.01$, compared to control pressor response
* $p<0.05$, compared to sham-operated rats

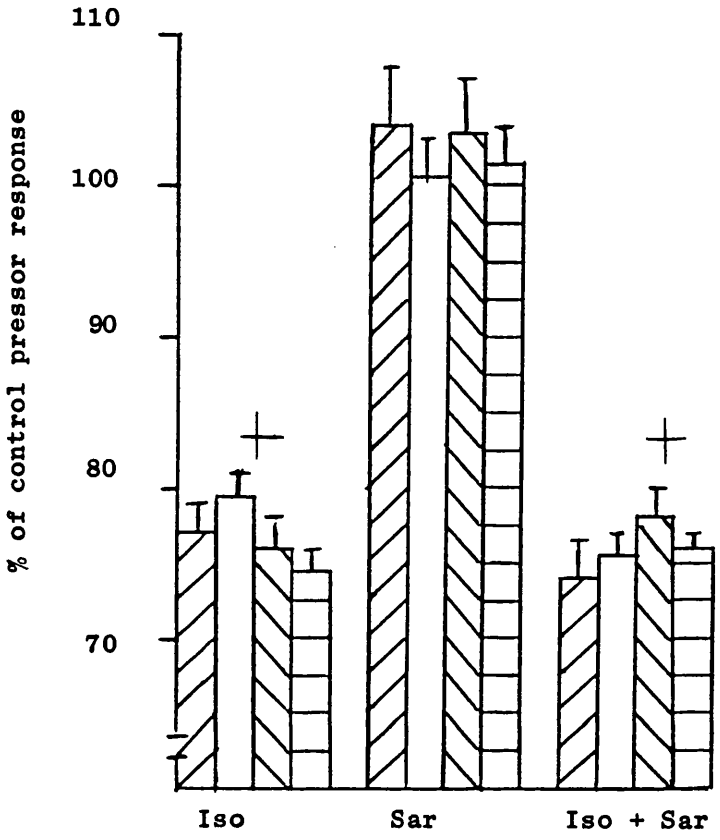


Fig 4.18

Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively) and male and female sham-operated rats (W_m, W_f respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group

W_mR W_m

W_fR W_f

+ $p<0.05$, groups in these sections significantly different to those in Sar section

Effect of ICI 118,551 on the angiotensin II potentiation of the pressor response to PNS and NA infusion

ICI 118,551 ($5x10^{-7}M$) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. Furthermore, ICI 118,551 had no significant effect on the angiotensin II induced potentiation of the pressor response to PNS and NA infusion in any of the preparations Figs 4.19 and 4.20.

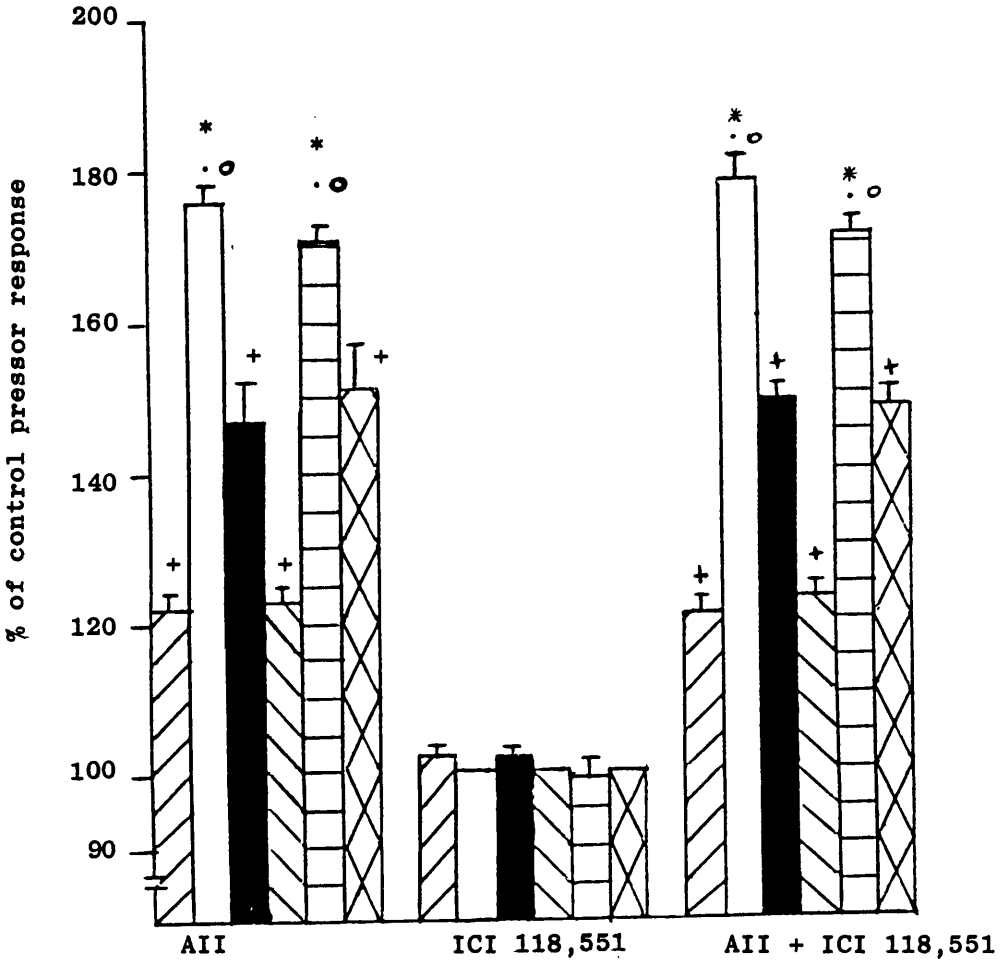


Fig 4.19

Effect of ICI 118,551 on the angiotensin II (AII)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham-operated rats (W_m, W_f respectively) and male and female renal hypertensive rats treated chronically with ICI 118,551 (W_mRI, W_fRI respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

W_mR W_m W_mRI

W_fR W_f W_fRI

+ $p<0.05$, compared to control pressor response
. $p<0.01$, compared to control pressor response
* $p<0.05$, compared to sham-operated rats
o $p<0.05$, compared to renal hypertensive rats treated chronically with ICI 118,551

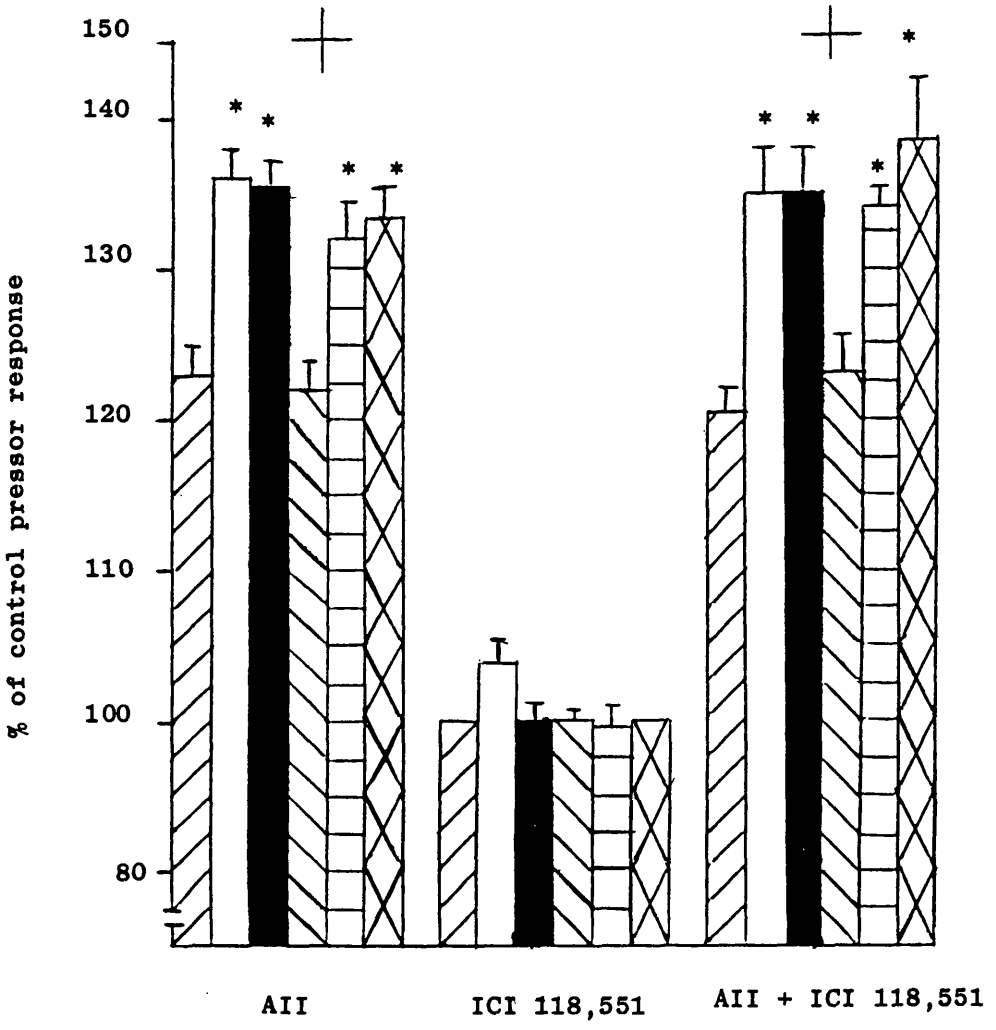


Fig 4.20

Effect of ICI 118,551 on the angiotensin II (AII)-induced potentiation of the pressor response to exogenous NA in the isolated perfused mesenteric vasculatures of male and female two-kidney, one-clip renal hypertensive rats (W_mR, W_fR respectively), male and female sham-operated rats (W_m, W_f respectively) and male and female renal hypertensive rats treated chronically with ICI 118,551 (W_mRI, W_fRI respectively).
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

W_mR W_m W_mRI

W_fR W_f W_fRI

* $p<0.05$, compared to sham-operated rats
┼ $p<0.05$, groups in these sections significantly different to control pressor response.

Discussion

The results described in this chapter suggest that physiological mechanisms facilitating sympathetic neurotransmission in the mesenteric vascular bed are enhanced in the renal hypertensive rats compared to the normotensive Wistars. Once again this facilitation seems to be quantitatively and qualitatively similar in male and female animals.

The results indicate that the facilitatory mechanisms are located presynaptically. This can be deduced from the comparison of the effects of isoprenaline and angiotensin II on the pressor response to PNS and NA infusion. It can be further concluded that the augmentation of presynaptic activity by isoprenaline is mediated via β_2-adrenoreceptors and that of angiotensin II via presynaptic angiotensin II receptors.

Chronic treatment of the renal hypertensive animals with ICI 118,551 significantly attenuated the degree of progression of the hypertension.

The results further indicate that the attenuation of this form of hypertension could be due to the presynaptic β_2-adrenoreceptor being antagonised by the chronic treatment with ICI 118,551.

It can also be inferred from the studies with cocaine and angiotensin II that chronic administration of ICI 118,551 in renal hypertensive animals caused a reduced release of neural NA.

CHAPTER 5

EXPERIMENTAL HYPERTENSION: OESTROGEN-INDUCED HYPERTENSION

5.1. Methods

5.1i Oestrogen treatment

Ethinyloestradiol was used for this study. Age matched (12-14 weeks old) male and female Wistar rats were used in this study. The ethinyl oestradiol (EO) treated groups received a subcutaneous injection of EO (1.5 mg/kg rat body weight) daily for three weeks. The age and sex matched controls received a subcutaneous injection of the vehicle, arachis oil, daily for three weeks.

For clarity in this chapter,

EO_m - EO treated male Wistar
EO_f - EO treated female Wistar
W_m - vehicle treated male Wistar
W_f - vehicle treated female Wistar

5.1ii Blood pressure measurements

Systolic blood pressure was monitored in the conscious animals by the tail-cuff method using a programmed electro-sphygmomanometer PE-300 (Narco bio-instruments) coupled to a flat-bed recorder (CR6505, J.J.Instruments). Systolic blood pressure was monitored at the end of each week of EO treatment. The mean of three readings on that day was taken as the systolic blood pressure.

The animals were weighed on the same day as the measurement of the systolic blood pressure.

5.1iii Isolated perfused tissues

Once the elevation of the systolic blood pressure had stabilized (after three weeks of EO treatment), the animals were sacrificed and the isolated perfused mesenteric vascular bed and the isolated perfused kidney preparations were set up as previously described in Section 2.2i. and Section 3.2i respectively.

Modified Kreb's solution (composition as described in Section 2.2i) was used as the perfusion medium for the mesenteric vascular preparation and Tyrode solution (composition as described in Section 3.2i) was used as the perfusion medium for the kidney preparation. The isolated mesenteric vascular preparation and the isolated kidney preparation were both taken from the same animal. The isolated kidney was cannulated and set up first, followed by the mesenteric vasculature from the same animal. Whilst the isolated kidney preparation was being set up care was taken to keep the mesenteric vasculature moist with the Kreb's solution. Not more than five minutes elapsed between the setting up of the isolated kidney preparation and the setting up of the isolated mesenteric vasculature.

The Kreb's and Tyrode solutions were perfused at a constant rate of 5 ml/min and 10 ml/min respectively by means of Watson Marlow peristaltic pumps. The perfusing solutions were aerated with a mixture of 95% oxygen and 5% carbon dioxide before passing through a warming coil maintained at 38°C. Changes in perfusion pressure were measured at a point close to the cannula by means of a pressure transducer (Bell and Howell, type 4-422-0001) and recorded on a Devices (M2) recorder.

After allowing time for the basal perfusion pressure to stabilize, usually 15 minutes, the preparations were subjected to either PNS or to a bolus of NA. PNS was delivered at 30Hz, 80V, 1 msec pulse width for 10 seconds duration for the mesenteric vascular preparation and 3Hz, 50V, 1 msec pulse width for 5 seconds duration for the kidney preparation.

The amount of NA given in the NA infusion was such that the pressor response elicited in the two different preparations was approximately similar to the PNS response in those preparations. The NA infusion was 0.1 ml of 10^{-6}M and 0.1 ml of 10^{-7}M of noradrenaline bitartrate solution for the mesenteric and kidney preparations respectively.

Once stable responses to PNS and NA infusion had been demonstrated, perfusion with other drugs began. The

method of perfusion of the drugs was as described previously in Section 2.2i.

Data was derived from the second response to PNS or NA infusion; the exception being experiments involving cocaine, when the first response was taken.

PNS and NA response data after drug perfusion in the preparations are expressed as a percentage of the control pressor response in order to standardize the data.

Age matched male and female Wistar rats (University of Bath strain) were used in this study. The animals used were supplied by the University of Bath Animal House.

5.1iv Statistical Analysis

Results were analysed using Student's t-test for group and paired mean comparisons.

Probability levels equal to or less than 0.05 were taken as indicating statistically significant differences. All comparisons employed the two-tailed test.

All data is taken from measurements and results from experiments after three weeks of chronic treatment with EO.

5.2 Results

5.2i Effect of chronic treatment with EO on the weight and systolic blood pressure of the animals

Figs 5.1 and 5.2 show the effect of chronic treatment with EO for three weeks on the weight and systolic blood pressure respectively.

As can be seen from Fig 5.1, both male and female rats treated chronically with EO lost weight significantly compared to control rats. The degree of weight loss in the male EO treated animals was significantly greater than that in the female EO treated animals; 17.69 $\pm$ 0.54% for the male EO treated and 12.28 $\pm$ 0.89% for the female EO treated animals ($p<0.001$).

The systolic blood pressure was also significantly higher in the EO treated groups compared to the control groups. Fig 5.2. This elevation remained stable at least up to four weeks of EO treatment when the treatment was continued further beyond the three weeks. The elevation of the blood pressure caused by the chronic treatment with EO was significantly higher in the female animals than in the male animals.

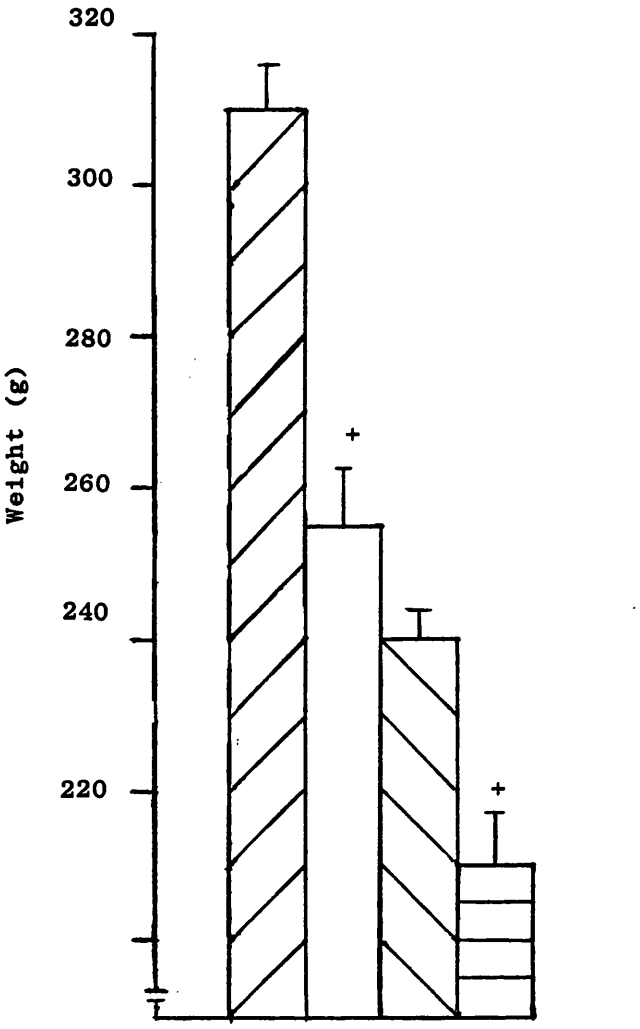


Fig 5.1

Effect of chronic treatment with ethinyloestradiol (EO) on the weights of Wistar rats.
Vertical lines indicate s.e. of mean.
n = 15 animals for each group.

EO-treated male Wistar rats

EO-treated female Wistar rats

Vehicle-treated male Wistar rats

Vehicle-treated female Wistar rats

+ $p<0.05$, compared to vehicle-treated rats.

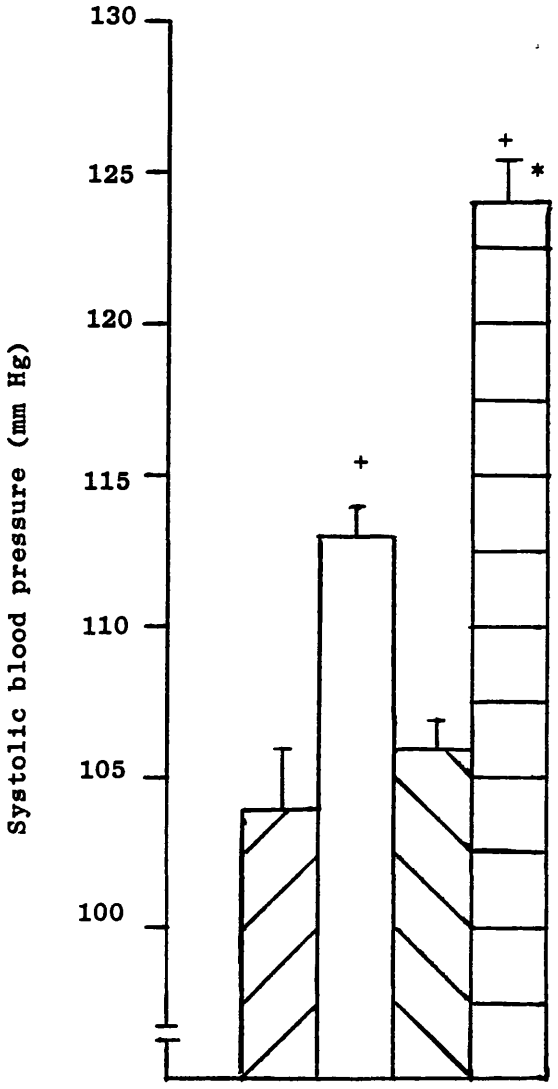


Fig 5.2

Effect of chronic treatment with ethinyloestradiol (EO) on the systolic blood pressure of Wistar rats.
Vertical lines indicate s.e. of mean.
n = 15 animals for each group.

EO-treated male Wistar rats

EO-treated female Wistar rats

Vehicle-treated male Wistar rats

Vehicle-treated female Wistar rats

+ $p<0.05$, compared to vehicle-treated rats.
* $p<0.05$, compared to EO-treated male rats.

5.2ii Effect of chronic treatment with EO on the pressor responses to PNS and NA infusion in the mesenteric and kidney preparations

Chronic treatment with EO significantly sensitized the mesenteric and kidney vasculature to NA in both the male and female animals. The pressor response to PNS in the animals chronically treated with EO was also enhanced compared to the control animals (vehicle treated). No significant differences in the degree of EO induced potentiation of pressor responses to PNS and NA infusion between the preparations from male and female animals were observed. Figs 5.3 and 5.4.

5.2iii Effect of chronic treatment with EO on the weight of the adrenal glands of the animals

Table 5.1 shows the mean wet weight of the adrenal glands of the animals used in this study.

The adrenal glands from the animals chronically treated with EO were found to be significantly heavier than those from the sex-matched controls, though no significant difference between the sexes of each group was found.

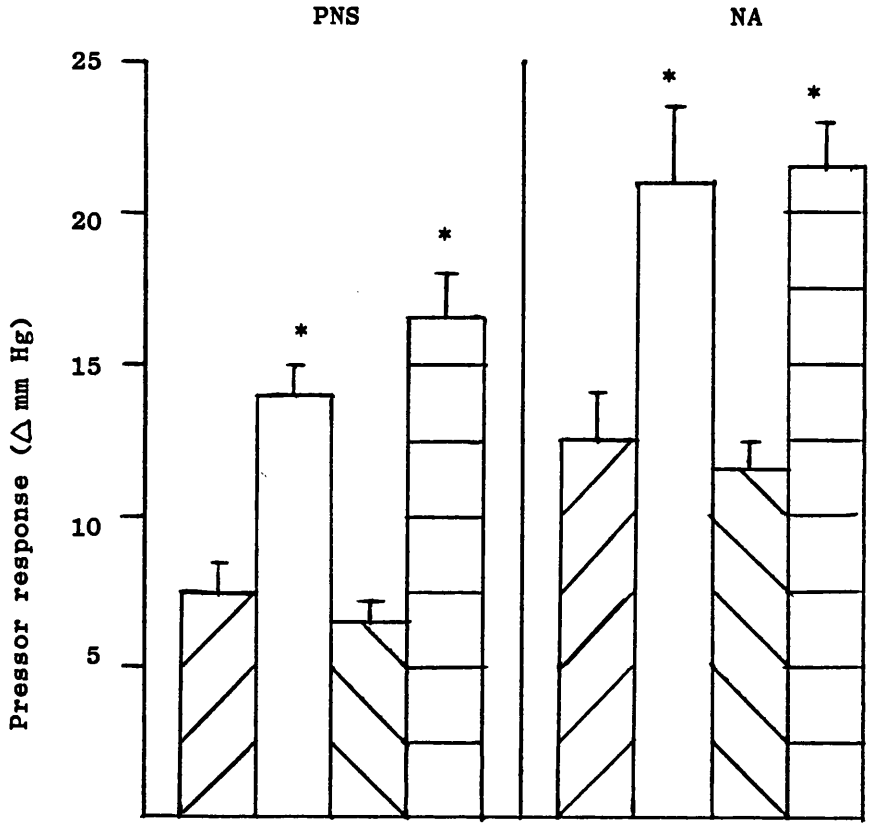


Fig 5.3

Pressor response to PNS and noradrenaline (NA) infusion in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean.
n = 15 animals for each group

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

* $p < 0.05$ compared to vehicle-treated rats

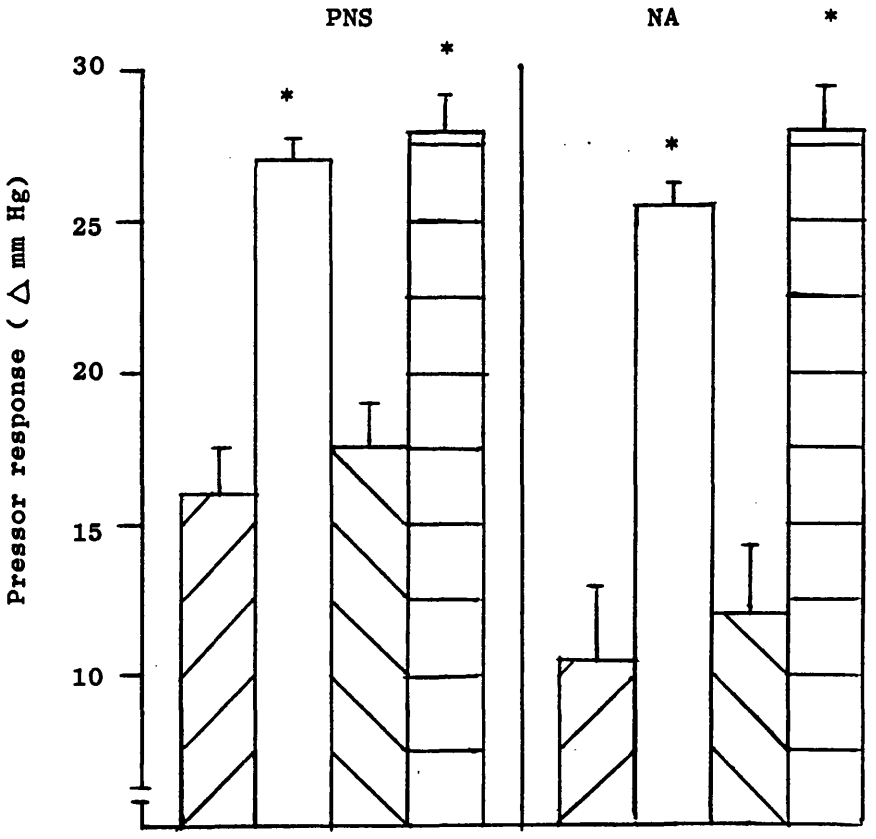


Fig 5.4

Pressor response to PNS and noradrenaline (NA) infusion in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle
Vertical lines indicate s.e. of mean.
n = 15 animals for each group.

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

* $p < 0.05$ compared to vehicle-treated rats

Table 5.1

Wet weight of adrenal glands from rats treated chronically with ethinyloestradiol (EO) and rats treated with arachis oil (vehicle)

	Wet weight of adrenal glands (mg)	
	Vehicle treated rats	EO-treated rats
Male rats	49.2 ± 3.4 (10)	+ 61.6 ± 3.1 (10)
Female rats	48.9 ± 3.1 (10)	+ 59.7 ± 2.8 (10)

Values are mean ± s.e. of mean
Number in parenthesis, number of animals.
+ $p<0.05$, significant differences from sex-matched vehicle treated rats.

5.2iv Effect of isoprenaline on the pressor responses to PNS and NA infusion in the rats chronically treated with EO

a) Mesenteric vascular bed

Isoprenaline ($5x10^{-8}M$) was used as the standard concentration in this study, and in following studies described in this chapter, as this concentration caused the greatest potentiation of the pressor response to PNS in the Wistar rats (Section 2.3ii).

Fig 5.5 shows the effect of isoprenaline on the pressor response to PNS and NA infusion in the preparations from rats treated with arachis oil and rats treated chronically with EO.

Isoprenaline caused a potentiation of the pressor response to PNS in the preparations from all the groups. This potentiation was however significantly greater in the preparations from animals treated chronically with EO than in those from the control animals.

There were no significant differences between the preparations from male and female animals from each group.

Isoprenaline caused an inhibition of the pressor response to NA infusion in all the preparations from all the groups. The degree of this inhibition was similar in all the preparations from all the animals.

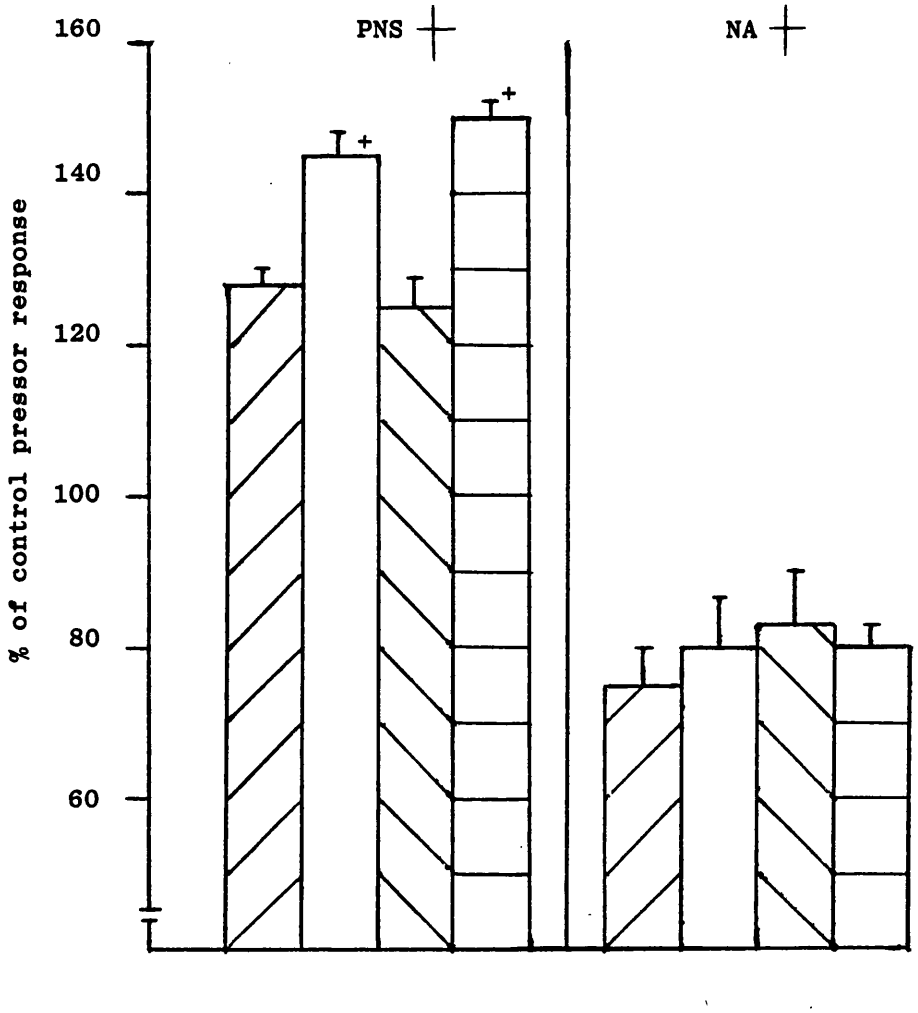


Fig 5.5

Effect of isoprenaline on the pressor responses to PNS and exogenous noradrenaline (NA) in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean.
n = 3 animals for each group.

EO-treated male Wistar rat

EO-treated female Wistar rat

Vehicle-treated male Wistar rat

Vehicle-treated female Wistar rat

$+$ $p<0.05$, all groups in these sections significantly different compared to control pressor response.
$+$ $p<0.05$, compared to vehicle-treated rats.

Isoprenaline alone had no significant effect on the basal perfusion pressure of any of the preparations from any of the groups.

b) **Kidney vasculature**

The standard concentration of isoprenaline used for the isolated kidney preparation in this and following studies described in this chapter was 10^{-8}M and 10^{-9}M for kidneys from male and female animals respectively, as these concentrations caused the greatest potentiation of the pressor response to PNS in the preparations from Wistar rats (Section 3.3ii).

Fig 5.6 shows the effect of isoprenaline on the pressor response to PNS and NA infusion in the preparations from animals treated with the vehicle and animals treated chronically with EO.

Isoprenaline alone had no significant effect on the basal perfusion pressure of the preparation from any of the animals, however isoprenaline significantly potentiated the pressor response to PNS in all the preparations from all the animals. This potentiation was significantly greater in the preparations from EO treated animals than that from the vehicle-treated animals. No significant differences in the potentiation of the PNS response between preparations from male and female rats were observed.

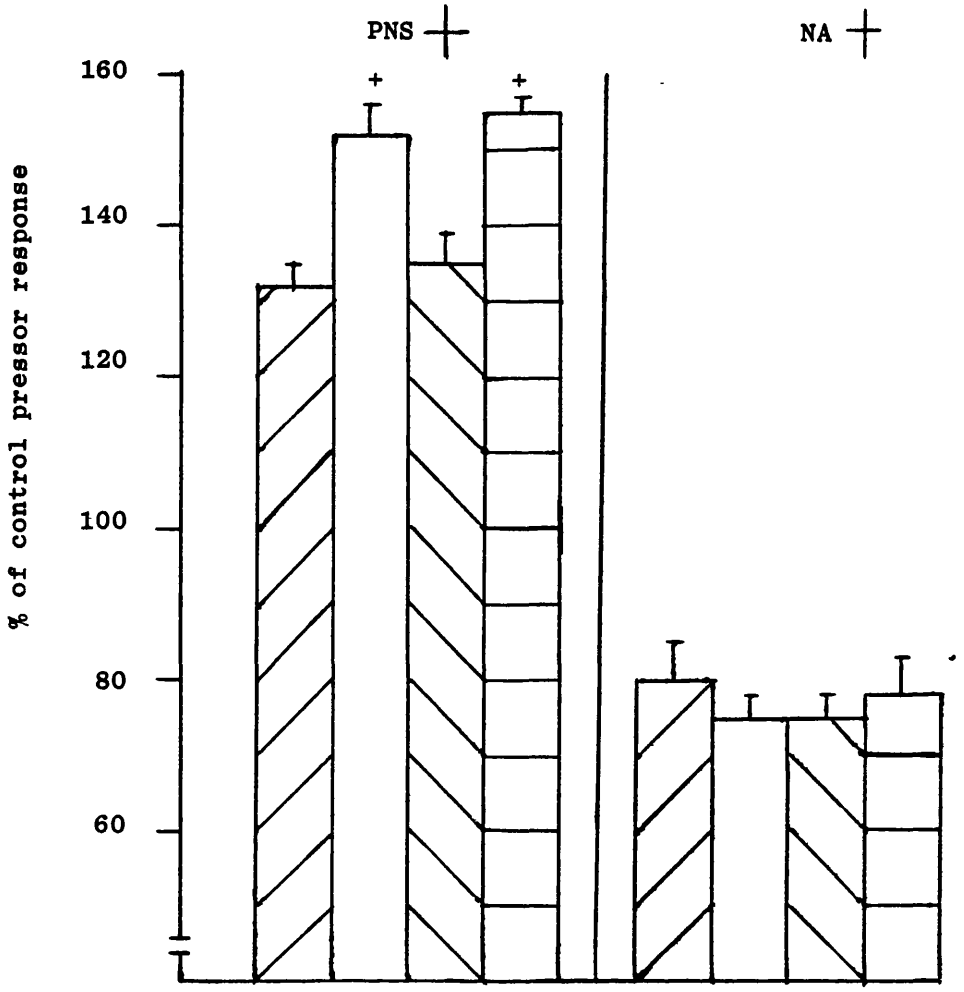


Fig 5.6

Effect of isoprenaline on the pressor responses to PNS and exogenous noradrenaline (NA) in the isolated perfused kidneys from rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle
Vertical lines indicate s.e. of mean.
n = 3 animals for each group

EO-treated male Wistar rats

EO-treated female Wistar rats

Vehicle treated male Wistar rats

Vehicle treated female Wistar rats

$+$ $p<0.05$ all groups in these sections significantly different compared to control pressor response.
+ $p<0.05$, compared to vehicle-treated rats.

Isoprenaline caused an inhibition of the pressor response to NA infusion in all the preparations from all the groups. The degree of this inhibition was similar in all the preparations.

5.2v Effect of ICI 118,551 on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

ICI 118,551 ($5x10^{-7}M$) alone had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. However, ICI 118,551 significantly abolished the isoprenaline induced potentiation of the pressor response to PNS in all the preparations.

ICI 118,551 ($5x10^{-7}M$) significantly reversed the isoprenaline induced inhibition of the pressor response to NA infusion in all the preparations. Fig 5.7 and Fig 5.8 for mesenteric and kidney preparations respectively.

5.2vi Effect of atenolol on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Atenolol ($10^{-7}M$) alone had no significant effect on either the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations.

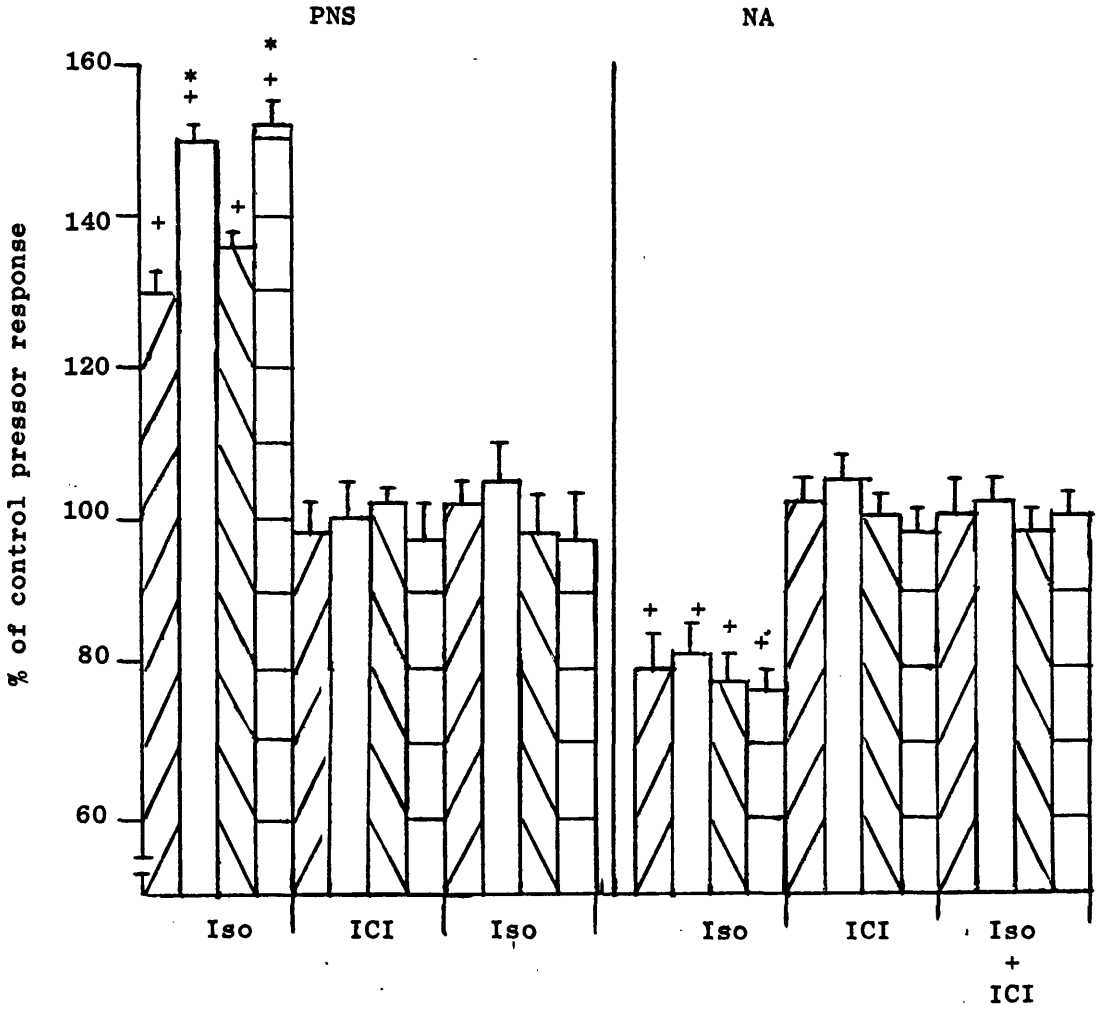


Fig 5.7

Effect of ICI 118,551 (ICI) on the isoprenaline (Iso)-induced effects on the pressor responses to PNS and noradrenaline (NA) infusion in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rats □ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

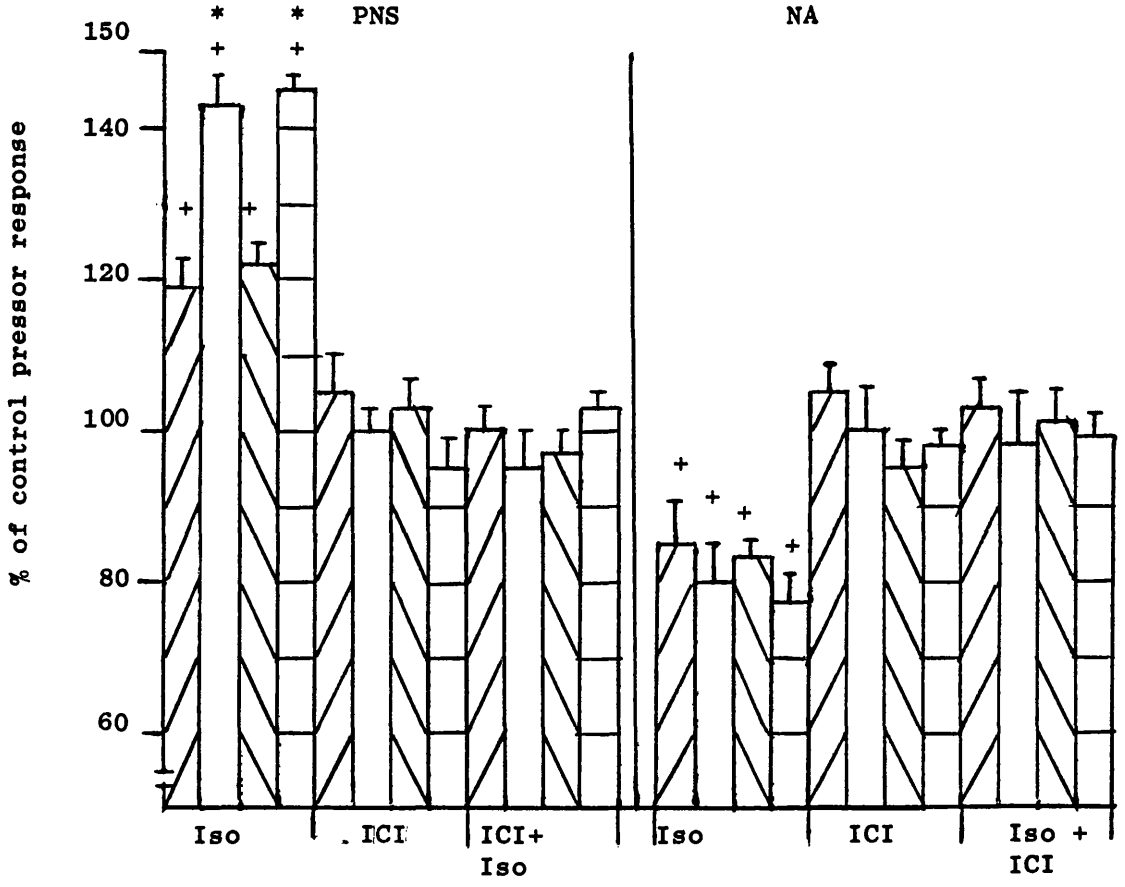


Fig 5.8

Effect of ICI 118,551 (ICI) on the isoprenaline (Iso)-induced effects on the pressor responses to PNS and noradrenaline (NA) infusion in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

Atenolol did not significantly affect the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations.

In all the preparations, atenolol significantly reversed the isoprenaline induced inhibition of the pressor response to NA infusion. Figs 5.9 and 5.10 for mesenteric and kidney preparations respectively.

5.2vii Effect of angiotensin II on the pressor responses to PNS and NA infusion

The concentrations of angiotensin II (AII) employed in this study were 10 ng/ml and 1ng/ml for the mesenteric and kidney preparations respectively. At the concentrations employed in this study, angiotensin II caused an initial transient elevation of the basal perfusion pressure (approximately 15%) in some of the preparations. This transient elevation of the basal perfusion pressure usually lasted for about 60 seconds before returning to its pre-angiotensin II perfusion levels. Further perfusion of angiotensin II caused no discernible effect on the basal perfusion pressure.

Table 5.2 shows the effect of NA infusion in the mesenteric and kidney preparations of animals treated chronically with EO and with vehicle in the presence of angiotensin II.

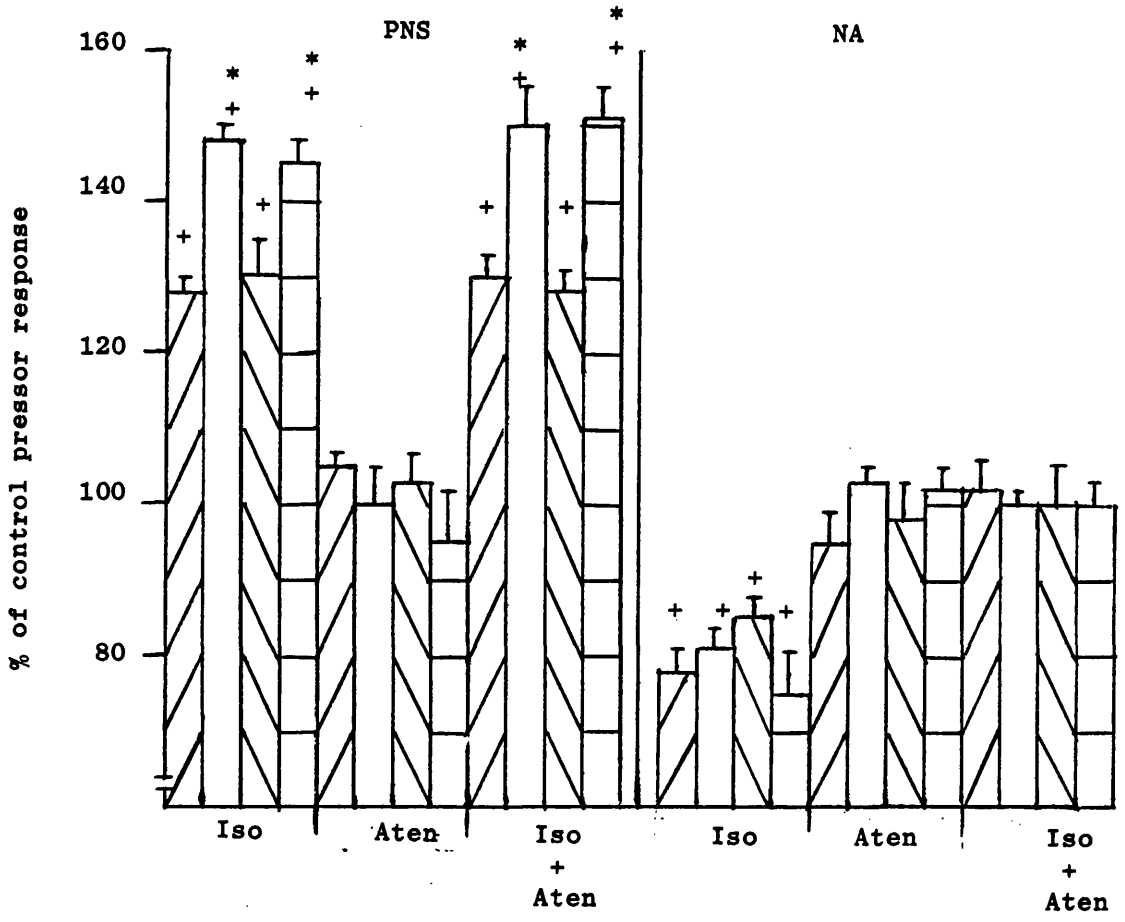


Fig 5.9

Effect of atenolol (Aten) on the isoprenaline (Iso)-induced effects on the pressor responses to PNS and noradrenaline (NA) infusion in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

E.O.-treated male rats ☐ Vehicle-treated male rats ⧄

EO-treated female rats ⧆ Vehicle-treated female rats ⧅

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

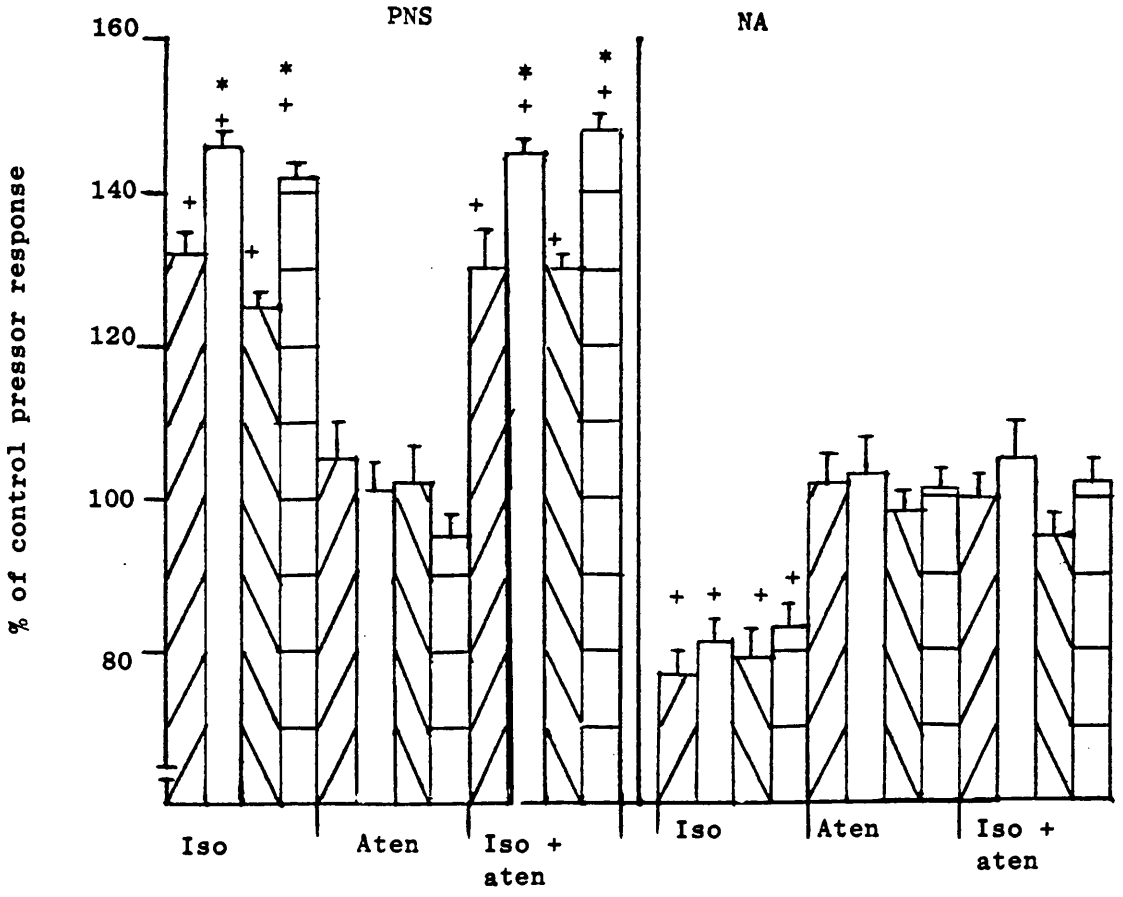


Fig 5.10

Effect of atenolol (Aten) on the isoprenaline (Iso)-induced effects on the pressor responses to PNS and noradrenaline (NA) infusion in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

Table 5.2

Effect of angiotensin II (AII) on the pressor response to noradrenaline (NA) infusion in the isolated mesenteric and kidney preparations from rats treated chronically with ethinyloestradiol (EO) and with vehicle

		A NA infusion (Δ mm Hg)	B AII + NA infusion (Δ mm Hg)	B/A x 100
MESENTERIC	EO-treated, male	14.3 ± 1.2	18.5 ± 1.5	129.4 ± 3.1
	Vehicle-treated, male	9.6 ± 1.5 *	11.9 ± 1.6 *	124 ± 1.6
	EO-treated, female	14.6 ± 1.4	18.3 ± 0.6	125.3 ± 2.4
	Vehicle-treated, female	9.8 ± 1.3 *	11.7 ± 0.9 *	119.4 ± 2.9
KIDNEY	EO-treated, male	25.1 ± 1.3	31.4 ± 1.6	125.1 ± 3.3
	Vehicle-treated, male	9.8 ± 1.4 *	12.1 ± 1.4 *	123.5 ± 1.6
	EO-treated, female	23.3 ± 1.7	29.6 ± 1.6	127 ± 2.3
	Vehicle-treated, female	10.1 ± 0.9 *	12.6 ± 1.3 *	124.7 ± 1.7

Values are mean ± s.e. of mean
n = 3 animals for each group
* $p < 0.05$ compared to sex-matched EO-treated rats

As can be seen from Table 5.2, angiotensin II caused a slight, but significant, potentiation of the pressor response to the NA infusion in all the preparations, but the degree of this potentiation was similar in all the preparations indicating that the EO treatment did not significantly affect the angiotensin II receptor.

Angiotensin II significantly potentiated the pressor response to PNS in the mesenteric and kidney preparations of all groups. The degree of this potentiation was found to be similar in the animals treated chronically with EO and with vehicle; thus indicating that EO treatment had no significant effect on the angiotensin II receptors.
Table 5.3.

No significant difference between the AII induced potentiation of the pressor response to PNS and NA infusion was found in any of the preparations from any of the groups.

5.2viii Effect of angiotensin II converting enzyme (ACE) inhibitor on the isoprenaline induced effects on the pressor response to PNS and NA infusion

Captopril ($5x10^{-6}M$) was used as the ACE inhibitor in this study. Captopril had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. However, captopril effectively inhibited the isoprenaline induced

Table 5.3

Effect of angiotensin II (AII) on the pressor response to PNS in the isolated mesenteric and kidney preparations from rats treated chronically with ethinyloestradiol (EO) and with vehicle

		A PNS during physiological saline perfusion (Δmm Hg)	B PNS during AII perfusion (Δ mm Hg)	B/A x 100
MESENTERIC	EO-treated, male	12.1 ± 1.3	15.4 ± 1.7	127.6 ± 2.1
	Vehicle-treated male	8.8 ± 1.6 *	11.1 ± 1.4 *	126.4 ± 1.8
	EO-treated, female	13.1 ± 1.6	17.2 ± 1.6	129.7 ± 1.4
	Vehicle-treated, female	9.6 ± 1.4 *	12.1 ± 1.7 *	125.9 ± 2.3
KIDNEY	EO-treated, male	20.8 ± 2.2	26.8 ± 2.4	129.1 ± 2.6
	Vehicle-treated, male	13.4 ± 1.6 *	17.3 ± 1.9 *	129.1 ± 1.8
	EO-treated, female	22.4 ± 1.9	27.5 ± 1.6	123.7 ± 2.8
	Vehicle-treated, female	15.1 ± 2.1 *	19.1 ± 1.8 *	125.5 ± 1.4

Values are mean ± s.e. of mean
n = 3 animals for each group
* $p<0.05$ compared to sex-matched EO-treated rats

potentiation of the pressor response to PNS (Figs 5.11 and 5.12 for mesenteric and kidney preparations, respectively) in all the preparations whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion (Figs 5.13 and 5.14 for mesentery and kidney preparations respectively) in any of the preparations.

5.2ix Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline induced effects on the pressor responses to PNS and NA infusion

Sar (200 ng/ml) by itself had no significant effect on the basal perfusion pressure or the pressor response to PNS and NA infusion in any of the preparations. Sar however effectively inhibited the isoprenaline-induced potentiation of the pressor response to PNS (Figs 5.15 and 5.16 for mesentery and kidney preparations respectively) in all the preparations, whilst not having any significant effect on the isoprenaline induced inhibition of the pressor response to NA infusion (Fig 5.17 and 5.18 for mesentery and kidney preparations, respectively) in any of the preparations from any of the groups.

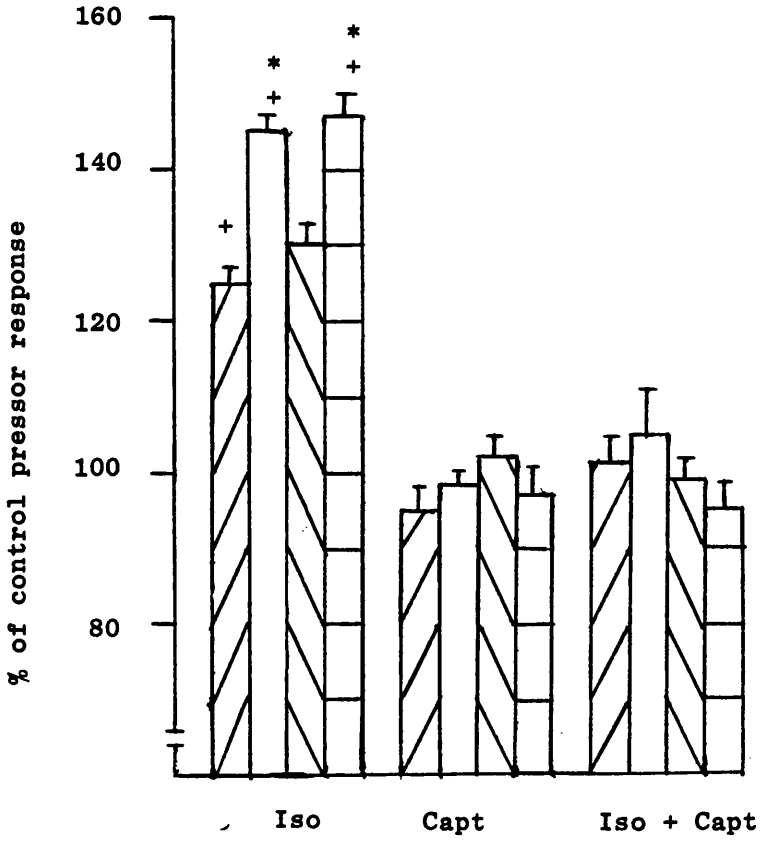


Fig 5.11

Effect of captopril (Capt) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group.

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

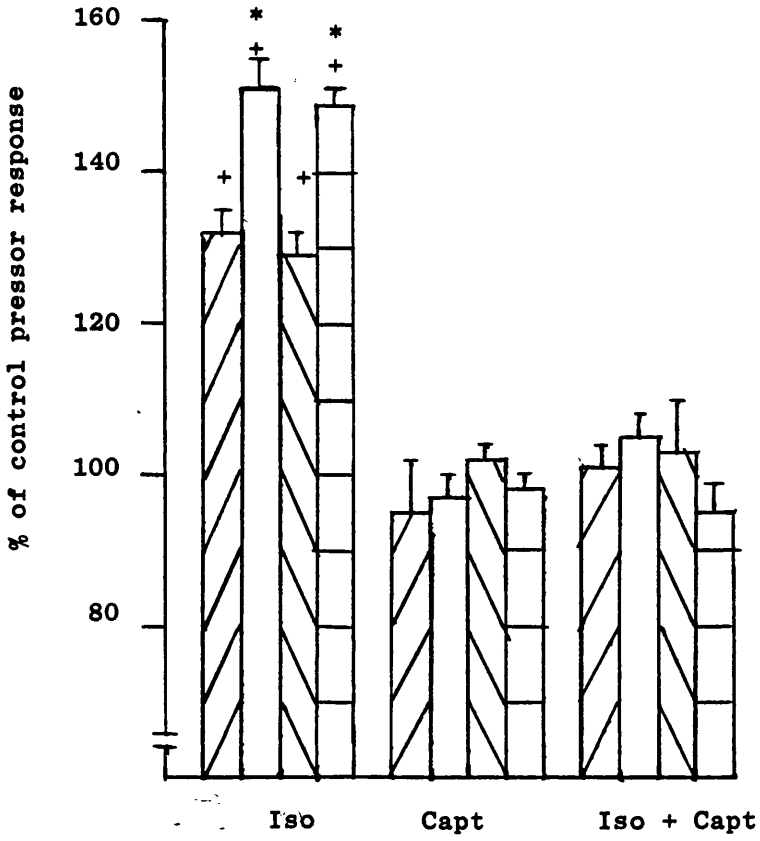


Fig 5.12

Effect of Captopril (Capt) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused kidney from rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group.

EO-treated male rats □ Vehicle-treated male rats ⊘

EO-treated female rats ⊟ Vehicle-treated female rats ⊘

+ $p<0.05$ compared to control pressor response and all other sections
* $p<0.05$ compared to vehicle-treated rats

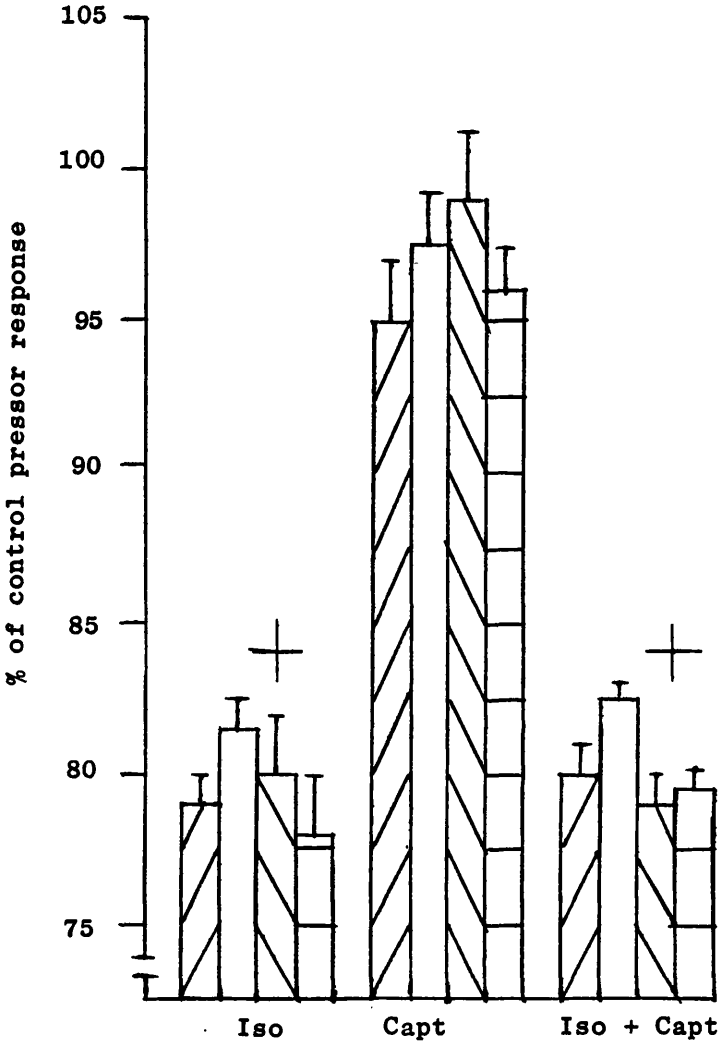


Fig 5.13

Effect of Captopril (Capt) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

EO-treated male rats ☐ Vehicle-treated male rats ▨

EO-treated female rats ▤ Vehicle-treated female rats ▧

+ $p<0.05$ significant difference between groups of these sections compared to control pressor response and all other sections.

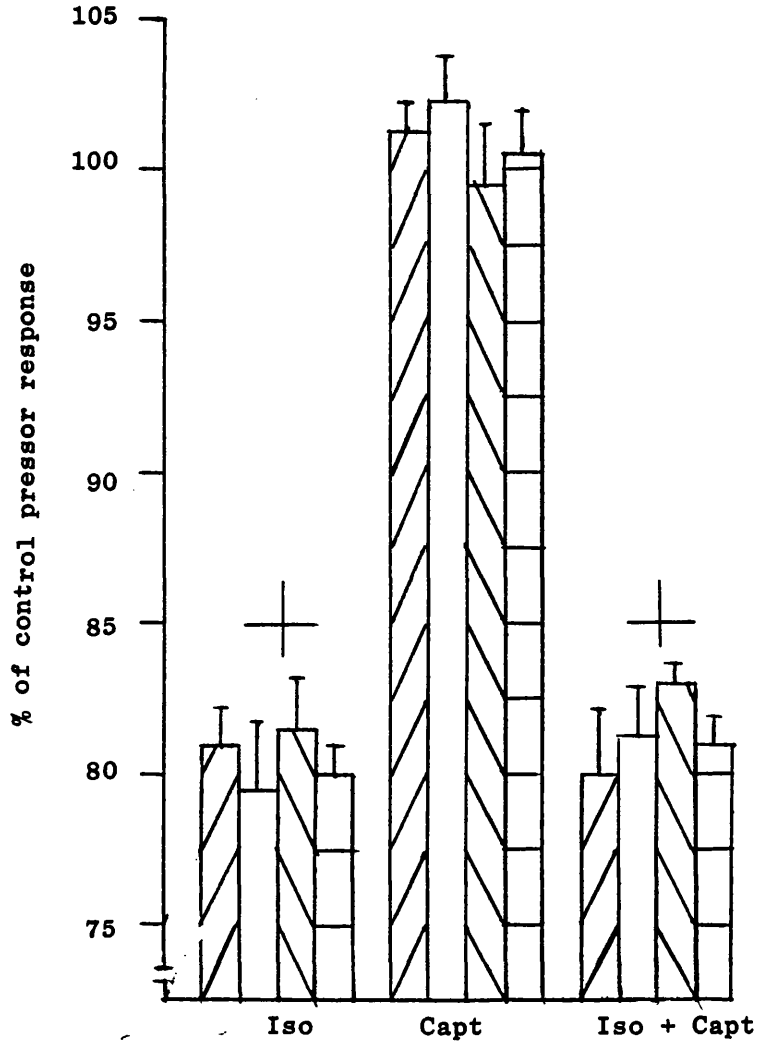


Fig 5.14

Effect of Captopril (Capt) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rat ☐ Vehicle-treated male rat ☒

EO-treated female rat ⊟ Vehicle-treated female rat ⊠

┼ $p < 0.05$, significant difference between groups in these sections compared to control pressor response and all other sections.

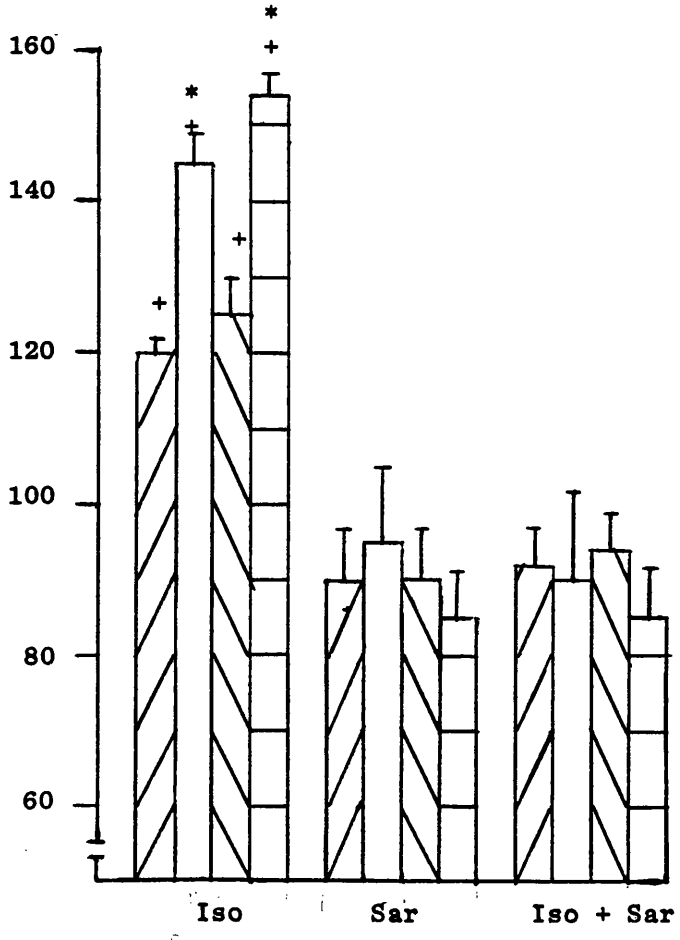


Fig 5.15
Effect of $[Sar^1\text{-}Ile^8]$ angiotensin II (Sar) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group.

EO-treated male rat ☐ Vehicle-treated male rat ▨

EO-treated female rat ▤ Vehicle-treated female rat ▧

+ $p<0.05$ compared to control pressor response and to groups in all other sections
* $p<0.05$ compared to vehicle-treated rats

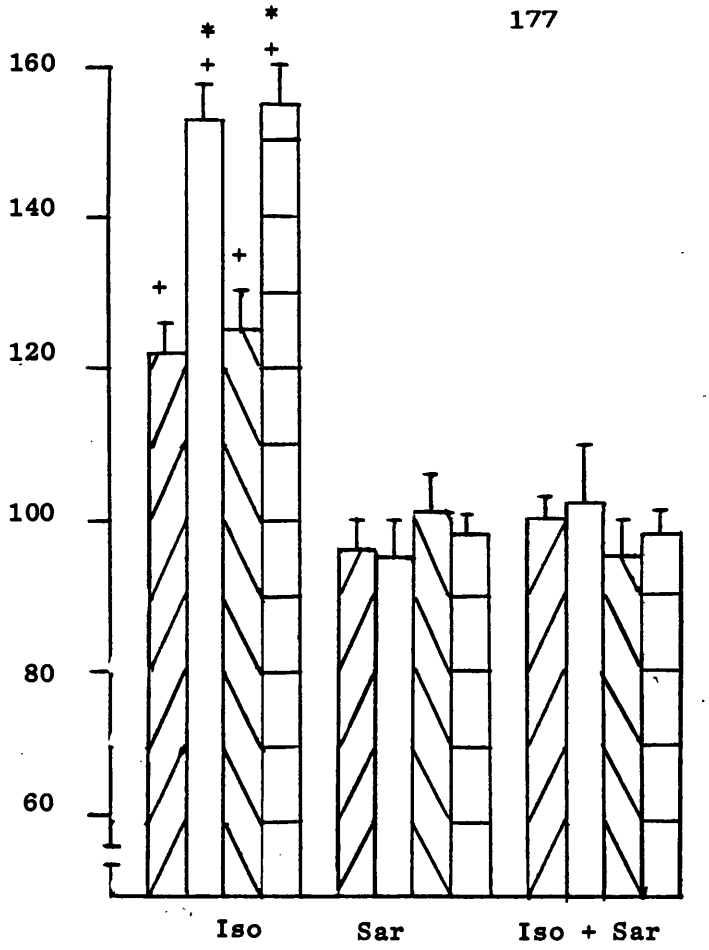


Fig 5.16
Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced potentiation of the pressor response to PNS in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rat ☐ Vehicle-treated male rat ⧄

EO-treated female rat ⊟ Vehicle-treated female rat ⧅

+ $p<0.05$ compared to control pressor response and to groups in all other sections
* $p<0.05$ compared to vehicle-treated rats

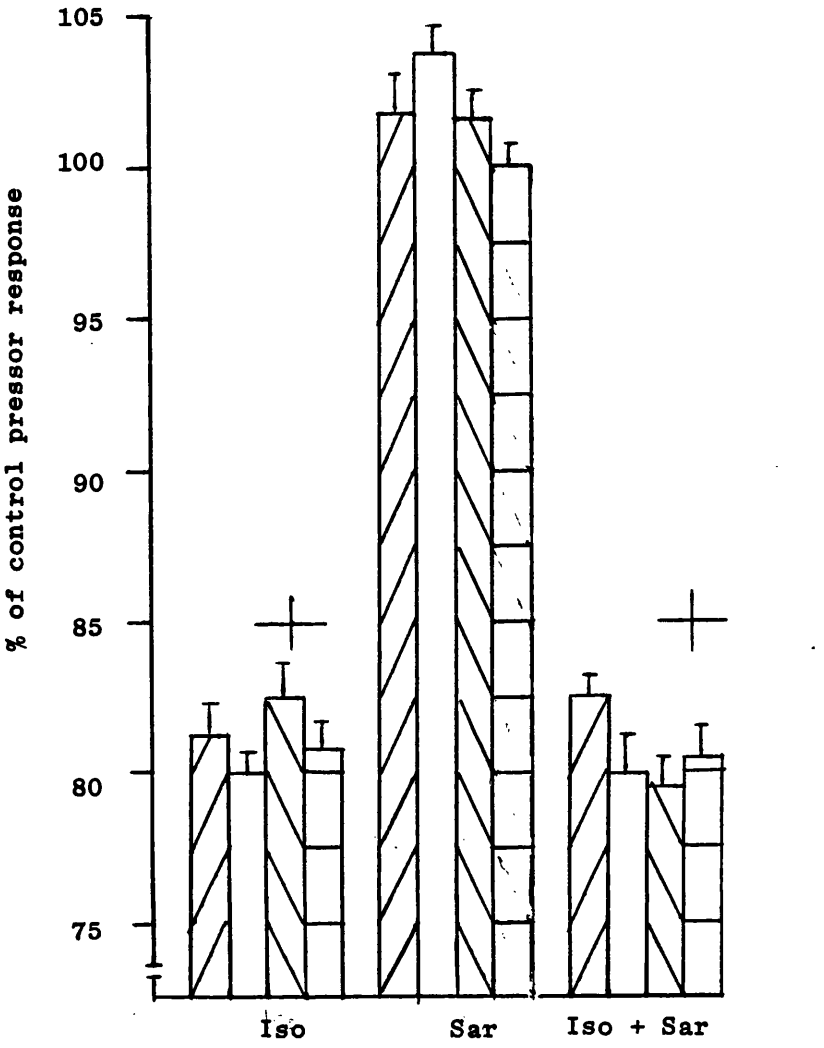


Fig 5.17

Effect of $[Sar^1\text{-}Ile^8]$ angiotensin II (Sar) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused mesenteric vasculature of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle.
Vertical lines indicate s.e. of mean. n = 3 animals for each group

EO-treated male rat □ Vehicle-treated male rat ▨

EO-treated female rat ▭ Vehicle-treated female rat ▧

+ $p < 0.05$, significant differences between groups in these sections compared to control pressor response and all other sections

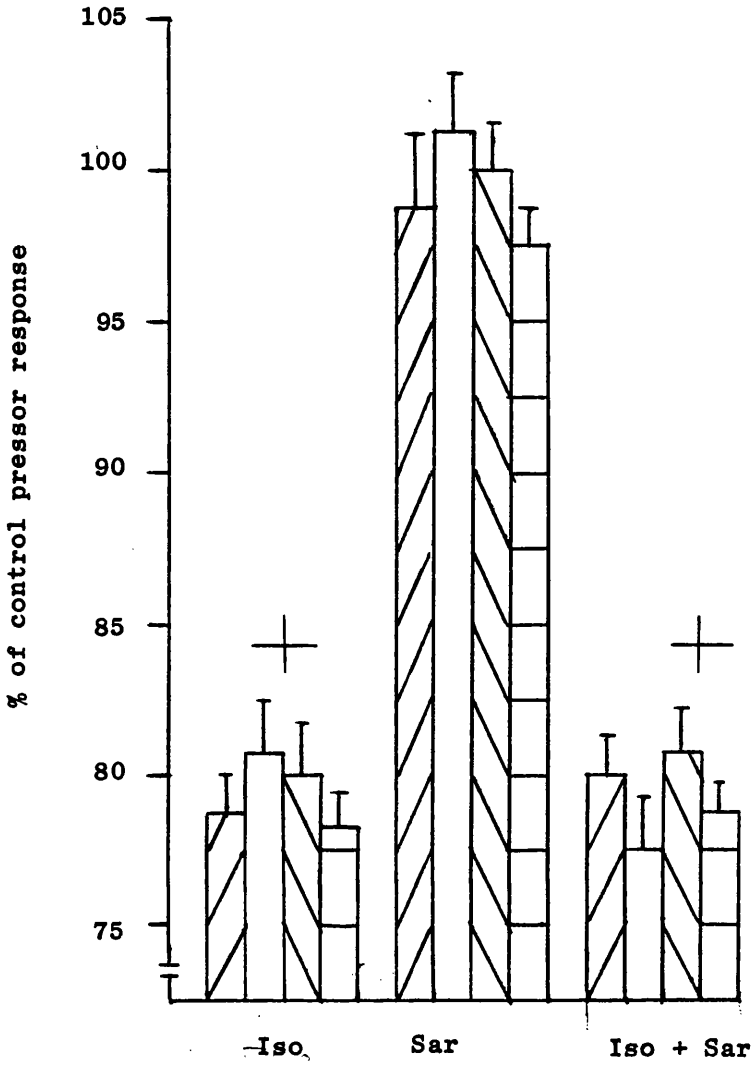


Fig 5.18

Effect of [Sar^1-Ile^8] angiotensin II (Sar) on the isoprenaline (Iso)-induced inhibition of the pressor response to exogenous NA in the isolated perfused kidney of rats treated chronically with ethinyloestradiol (EO) and rats treated with vehicle. Vertical lines indicate s.e. of mean. n = 3 animals for each group.

EO-treated male rat ☐ Vehicle-treated male rat ☐

EO-treated female rat ⊟ Vehicle-treated female rat ⧅

+ $p<0.05$, significant differences between groups in these sections compared to control pressor response and all other sections.

Discussion

The results indicate that chronic treatment with EO induces hypertension in rats and that this is greater in female rats than in male rats.

Chronic administration of EO also causes a weight reduction in the animals, the effect being greater in male rats than in female rats.

The significant increase in weight of the adrenal glands in the EO treated animals could be associated with the hypertrophic appearance of the gland in the EO treated animal compared to the vehicle treated animals.

Increased vascular sensitivity to NA of perfused mesenteric beds and kidney preparations was observed after chronic EO treatment. The EO treatment, however, did not affect the vascular sensitivity to angiotensin II or the postsynaptic sensitization of adrenergic receptors by angiotensin II.

Chronic treatment with EO seemed to augment physiological mechanisms facilitating sympathetic neurotransmission in the mesentery and kidney vasculature. This facilitation seems to be qualitatively and quantitatively similar in male and female rats. That the facilitatory mechanism is located presynaptically can be inferred from the comparison of the effects of isoprenaline on the pressor

response to PNS and NA infusions. It can be further concluded that the augmentation of presynaptic activity by isoprenaline is mediated via β_2-adrenoreceptors. Chronic treatment with EO enhanced the presynaptic β_2-adrenoreceptor activity because the isoprenaline further enhanced the pressor response to PNS in the animals treated chronically with EO compared to the animals treated with arachis oil. However, no increased activity or sensitivity of the pre-synaptic angiotensin II receptor was noted in relation to the chronic treatment with EO.

Chapter 6

PLASMA NORADRENALINE AND ADRENALINE LEVELS: ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

6.1 Introduction

This chapter is devoted to the quantification of circulating plasma NA and Ad levels in all the strains and types of rats used.

Firstly, the plasma catecholamine levels were measured at different ages (3 weeks to 15 weeks) in the SH and Wistar rat in order to ascertain if there were any differences, or similarities, between the two strains and between the sexes of each strain.

The catecholamine level was also measured in the "Goldblatt two-kidney, one-clip" renal hypertension model and compared with the SH and control Wistar animals. The age of the rats used in this study was 12 weeks to 15 weeks.

As shown in Chapter 4, chronic ICI 118,551 (25 mg/kg p.o. daily) treatment tended to reduce the degree of hypertension in the renal hypertensive model. Therefore the catecholamine levels were also measured in the chronic ICI 118,551 - treated renal hypertensive models.

Chronic ethinyloestradiol (EO) treatment tended to increase the blood pressure of the Wistar rats (see Chapter 5) and therefore the effect of the chronic EO treatment on plasma NA and Ad levels was investigated. Chronic EO treatment consisted of a subcutaneous injection

of EO, 1.5mg/kg, daily for three weeks. Wistar rats aged twelve to fourteen weeks were used for this study.

In all experiments, the blood samples from the animals were obtained by cardiac puncture under ether anaesthesia.

6.2 Methods

6.2i Collection of blood samples

Blood samples for catecholamine analysis were collected by cardiac puncture. Approximately 4ml of blood was taken from each animal and put into chilled glass tubes containing 100ul of 0.2M reduced glutathione (GSH, Sigma) and 100ul of heparin (1000 U/ml). The blood was centrifuged at 2000 r.p.m. for 10 minutes at 4°C (MSE, Chillspin) to separate the plasma phase, and the plasma samples were stored frozen at -25°C until assayed.

6.2ii Determination of plasma catecholamines

Plasma samples were assayed for adrenaline (Ad) and noradrenaline (NA) content by a modification of the method described by Eriksson and Persson (1982), utilizing alumina extraction and analysis of the extracts by high performance liquid chromatography (HPLC).

6.2iii Preparation of alumina (aluminium oxide)

Aluminium oxide was activated in order to obtain the grade recommended by Anton and Sayre (1962). 100 grammes of aluminium oxide (Brockmann grade, neutral, BDH) was added to 500 ml of 2M HCl in a beaker, covered and heated to 90°-100°C on a hot plate. The temperature was monitored with a thermometer and the aluminium oxide was

continuously stirred with a magnetic stirrer for 45 minutes at this temperature. The aluminium oxide was then allowed to settle and the yellow supernatant fluid was discarded along with the finer alumina particles.

The precipitate was washed twice with fresh 250ml portions of 2M HCl at 70°C for 10 minutes, discarding the supernatant each time. In the final acid wash, the alumina was stirred in 500ml of 2M HCl at 50°C for 10 minutes. After decanting the HCl, the precipitate was repeatedly washed (20 times) with fresh 200ml volumes of distilled water until the supernatant had a pH of 3.4.

Finally, the aluminium oxide was transferred to an evaporating dish and heated at 120°C for 1 hour and at 200°C for a further 2 hours in an oven. The alumina was then stored in a capped glass bottle in a dessicator at room temperature.

6.2iv Extraction procedure

1.5ml portions of plasma samples were placed in Eppendorf tubes to which 50 pmoles of the internal standard, 3,4-dihydroxybenzylamine (DHBA , Sigma) dissolved in 0.2M perchloric acid, had been added. The samples were well mixed and transferred to plastic tubes containing 50µl of 50mM reduced glutathione (GSH, Sigma), 50µl of 300mM ethylenediaminetetra-acetic acid disodium salt (EDTA,BDH) and 25mg of the activated alumina. 0.2ml of 1M Tris

buffer (pH 8.6), prepared by mixing 1M solutions of Trizma base and Trizma hydrochloride (both from Sigma), was then added to the samples. The sample tubes were capped, manually shaken and mixed on a Luckham rotary mixer for 20 minutes.

After the mixing, the tubes were centrifuged at 1000 r.p.m. for 5 minutes (IEC Centra-7) to settle the alumina and the supernatant was discarded. The alumina was washed 3 times with a 3mM EDTA solution (pH 7.0), centrifuging between washes and discarding the supernatant. After the final wash with the 3mM EDTA the samples were centrifuged and the supernatant discarded. 200µl of 0.2M perchloric acid was then added to the alumina, mixed for 10 minutes and the sample tubes centrifuged at 100 r.p.m. for 5 minutes. 150µl of the supernatant was then pipetted out and stored frozen at -25°C until HPLC analysis.

6.2v Assay of plasma extracts for catecholamines by high performance liquid chromatography (HPLC)

The adrenaline (Ad) and noradrenaline (NA) contents of the perchloric acid extracts were determined by reversed phase, ion-pair HPLC with electro-chemical detection.

The liquid chromatograph was composed of an LDC Model III Constametric pump, a Rheodyne 7125 injection valve with a 50ul loop, a 25 cm x 4.6 mm i.d. stainless steel analytical column packed with 5 um diameter Spherisorb-ODS

particles (Anachem) and a BAS LC-4A amperometric detector. The detector was operated at +0.65v with an Ag/AgCl reference electrode (BAS RE-1) and a glassy carbon working electrode. The flow rate of the mobile phase was kept constant at 1.0ml/min.

The mobile phase consisted of an acetate/citrate buffer (pH 5.2) containing 5% methanol (HPLC grade, Fisons) and 0.5mM 1-octanesulphonic acid sodium salt (HPLC grade, Fisons), the ion pairing agent.

The composition of the acetate/citrate buffer was (mM):

Sodium acetate, 100; Sodium hydroxide, 60; citric acid, 40.

The water used for the mobile phase was deionized and filtered by a Millipore Milli-Q water purification system. Prior to use, the mobile phase was filtered under vacuum and degassed by vigorously bubbling helium through the solution for 20 minutes.

Chromatograms were recorded on a JJ Instruments flat-top recorder. Typical chromatograms from a plasma extract and a non-extracted standard solution are shown in figure 6.1. Peak heights were measured manually and peak height ratios (NA/DHBA and Ad/DHBA) calculated.

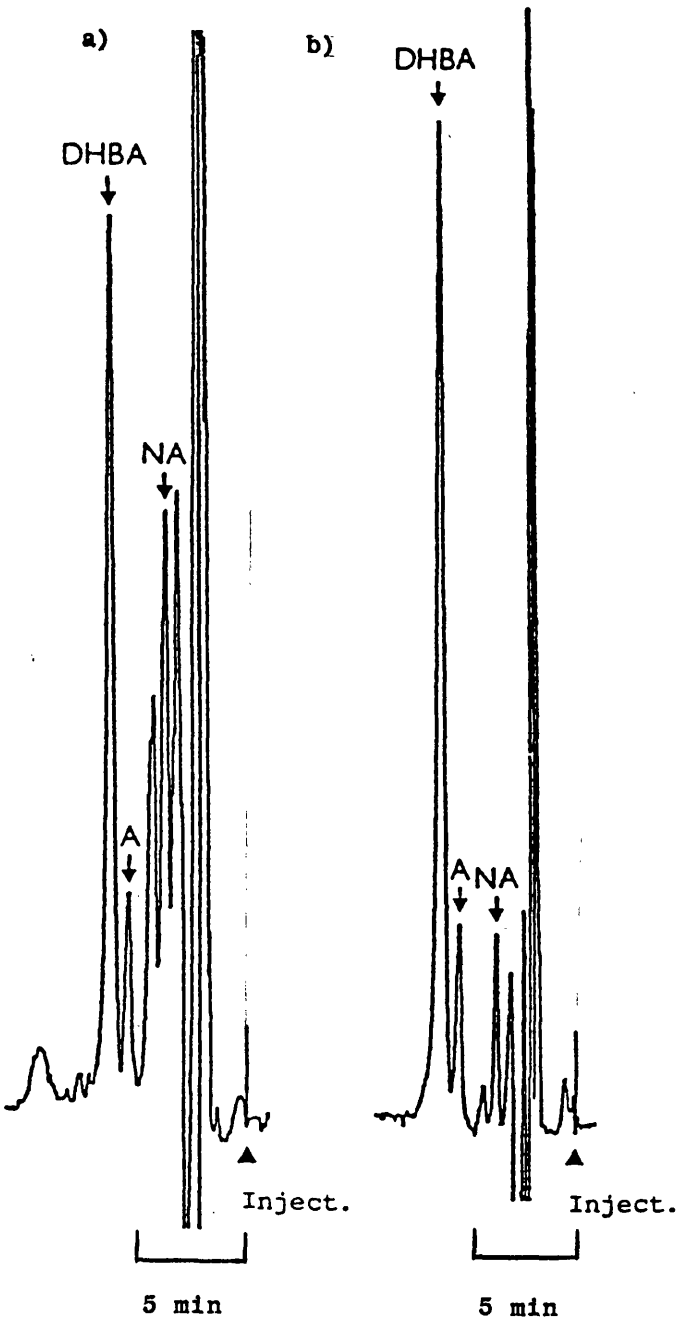


Fig 6.1. Typical examples of chromatograms from

a) extracted plasma sample
b) non-extracted standard sample

A= Adrenaline, NA= Noradrenaline,
DHBA= 3,4-Dihydroxybenzylamine.

6.2vi Calibration curve

Aliquots of 50 pmoles of DHBA in 0.2M perchloric acid were added to 1.5ml portions of known increasing concentrations of NA and Ad in the Eppendorf tubes and the samples underwent the extraction procedure previously described in section 6/2 iv.

The peak height ratios (NA/DHBA and Ad/DHBA) were plotted against the catecholamine concentrations to obtain calibration curves for both Ad and NA. Typical calibration curves are shown in Fig 6.2. These curves were used to convert peak height ratios to concentration values (pmol/ml) when analysing the plasma samples.

6.2vii The linearity of the detector response and the extraction procedure

The linearity of the detection system was evaluated by injecting standard solutions directly into the chromatographic system. The solutions contained increasing amounts of adrenaline and noradrenaline and a fixed concentration of DHBA in 0.2M perchloric acid.

The linearity of the extraction procedure was tested by putting increasing concentrations of NA and Ad samples through the extraction procedure. The internal standard, DHBA, was not added to the samples at the beginning of the extraction procedure, but was added in the 0.2M perchloric

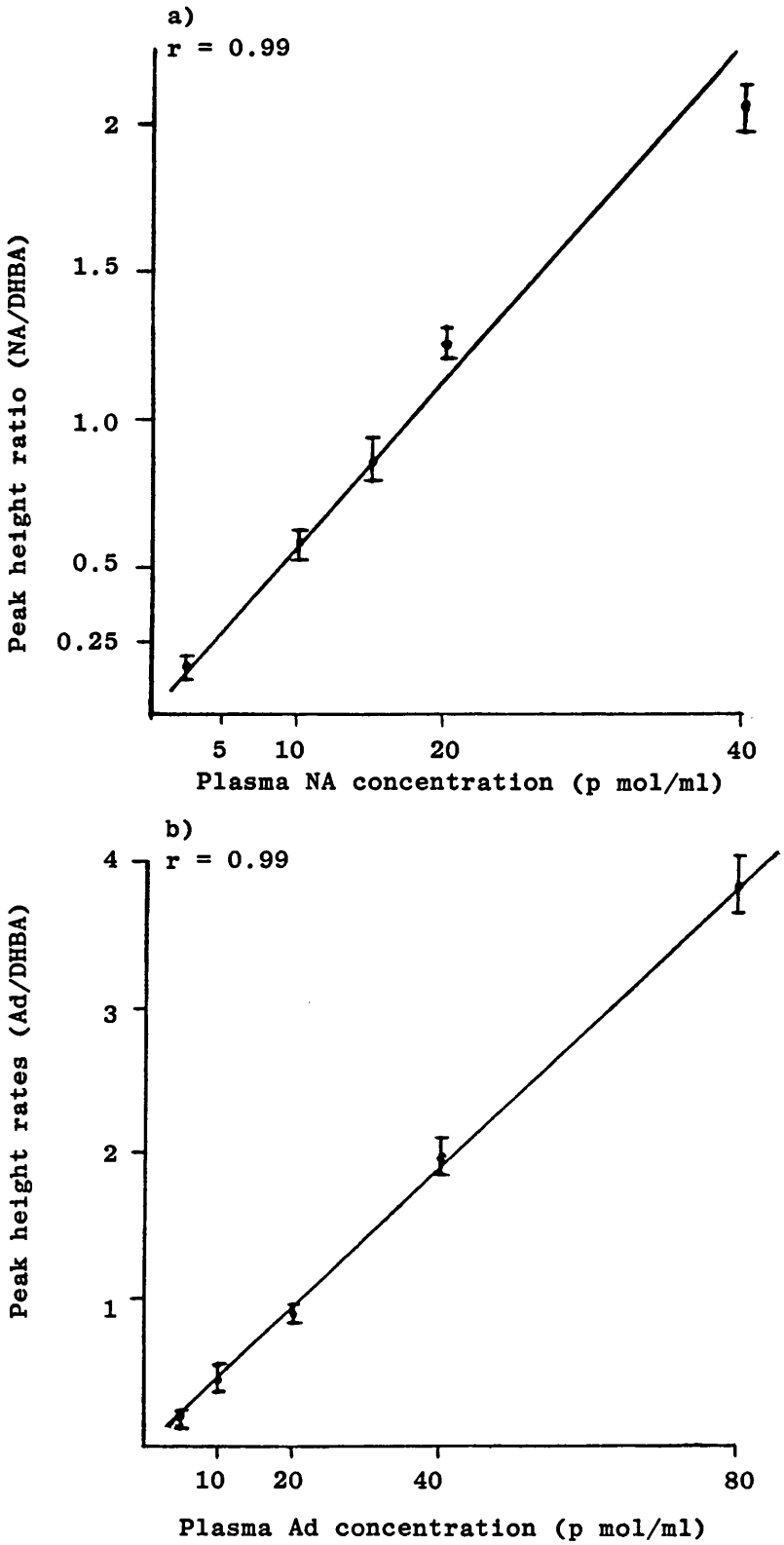


Fig 6.2 Caliberation curves for (a) noradrenaline (NA), and (b) adrenaline (Ad). DHBA = 3,4-dihydroxybenzylamine. Each point is the mean of three determinations. Vertical lines indicate s.e. of mean.

acid used for the final elution, at the appropriate concentration. The extracts were then analysed by HPLC and the peak height ratios calculated.

Both the detector response and the extraction procedure were found to be linear within the range corresponding to plasma concentrations of 2.5-40 pmol/ml for noradrenaline and 5-80 pmol/ml for adrenaline (r 0.99).

6.2viii Calculation of recovery

The recovery of noradrenaline and adrenaline was calculated by comparing the peak height ratios from the alumina extraction of spiked catecholamine samples with the peak height ratios from direct injections of the same concentrations of catecholamines into the chromatogram. The recovery of DHBA was calculated by comparing the peak height ratios obtained during the construction of calibration curves with those obtained when the DHBA was added during the final elution.

The recoveries for adrenaline, noradrenaline and DHBA were found to be 57%, 57% and 58% respectively.

6.3 Results

6.3i Effect of age on the plasma NA and Ad levels in the SH and Wistar rat.

Figs 6.3 and 6.4 show the plasma levels of NA and Ad, respectively, in the SH and age matched control Wistar animals.

At the ages studied in this experiment, the SH animals had significantly higher plasma NA and Ad levels than their respective Wistar controls. No significant differences in the plasma catecholamine levels between the male and female animals of each strain was found.

6.3ii Effect of renal hypertension on plasma NA and Ad levels

Wistar male and female animals were used.

Renal hypertension was induced by the two kidney, one clip method as previously described in Chapter 4. After the animals attained a state of established elevated blood pressure, usually after 12 weeks, they were anaesthetized with ether and the blood was obtained by cardiac puncture.

Table 6.1 shows the plasma levels of NA and Ad in the renal hypertensive animals and control sham operated animals.

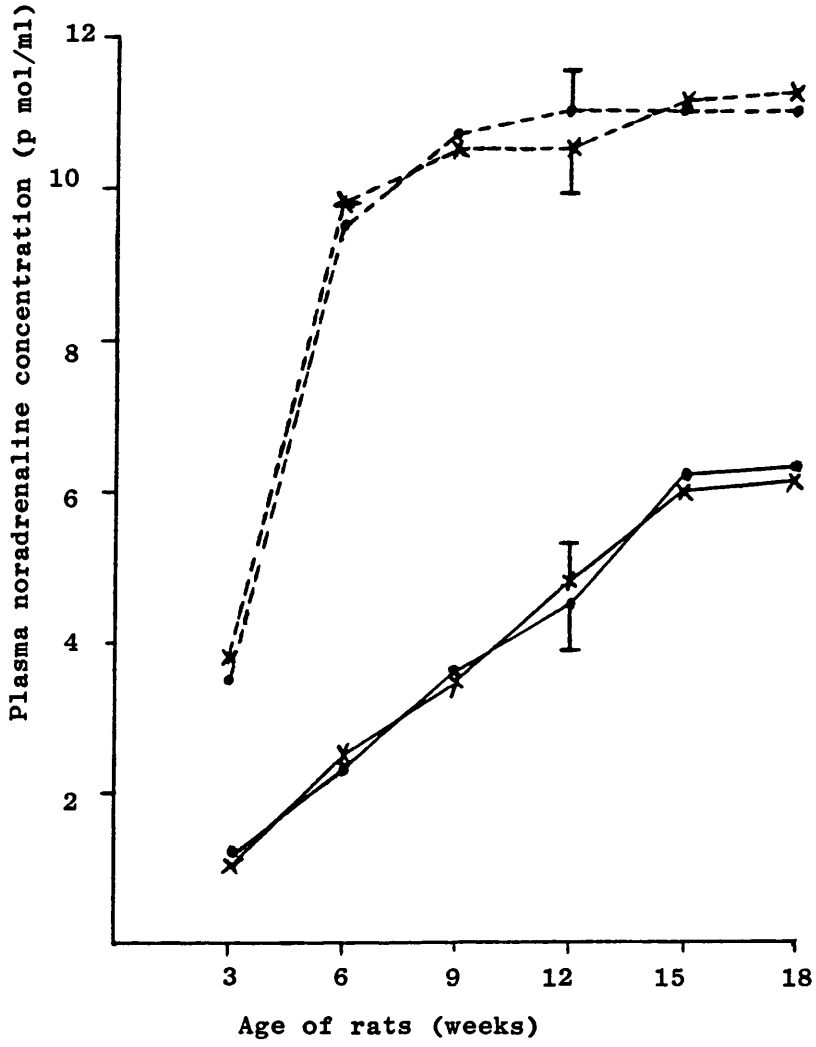


Fig 6.3. Effect of age on the plasma NA levels in the male SH (....), female SH (x...x), male Wistar (•——•) and female Wistar (x—x) rats. for each point. Vertical lines indicate s.e. of mean (most omitted for clarity). n = 8 animals.
At all points, plasma NA levels in SH rats significantly greater than that in Wistar rats ($p<0.05$)

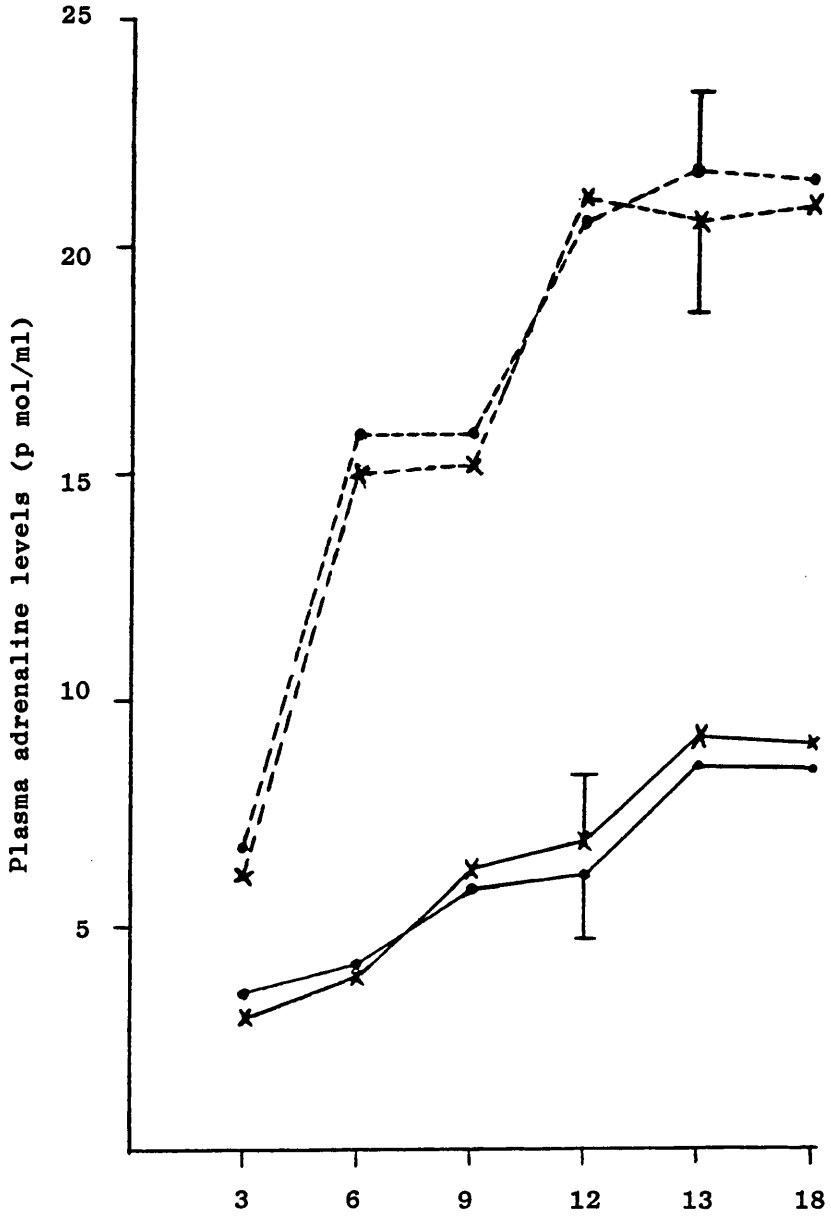


Fig 6.4 Effect of age on the plasma Ad levels in the male SH (•---•), female SH (x---x), male Wistar (•——•) and female Wistar (x——x) rats.
Vertical lines indicate s.e. of mean (most omitted for clarity). n = 8 animals for each point.
At all points, plasma Ad levels in SH rats significantly greater than that in Wistar rats (p 0.05).

Table 6.1

Plasma levels of NA and Ad in renal hypertensive rats and control sham operated rats

	Plasma NA concentration (pmol/ml)	Plasma Ad concentration (pmol/ml)
Renal hypertensive male Wistar rat	12.8 ± 0.4	23.3 ± 0.7
Control male Wistar rat	4.3 ± 0.6	7.7 ± 1.3
Renal hypertensive female Wistar rat	12.8 ± 0.3	24.6 ± 0.5
Control female Wistar rat	4.4 ± 0.5	7.2 ± 1.4

Values are given as mean s.e. of mean n = 10 animals for each group.

Plasma NA and Ad levels significantly greater in renal hypertensive rats than that in sham operated rats ($p<0.01$)

The plasma levels of NA and Ad were significantly elevated ($p<0.01$) in the renal hypertensive animals compared to the control animals. This elevation was found to be significantly greater ($p<0.05$) than the plasma NA and Ad levels in the sex and age matched SH animals. Table 6.2.

6.3iii Effect of chronic ICI 118,551 treatment on the plasma NA and Ad levels in the renal hypertensive animals

The plasma levels of NA and Ad in the chronically ICI 118,551–treated renal hypertensive animals are shown in Table 6.2. The plasma catecholamine levels were not significantly different from those in the sex- and age-matched SH animals but significantly lower than those of the untreated renal hypertensive animals.

No significant difference in the plasma catecholamine level was observed between the male and female of each strain of animal.

Table 6.2

Plasma NA and Ad levels in male and female SH rats (SHR_m and SHR_f, respectively), male and female renal hypertensive rats (W_mR and W_fR, respectively) and male and female renal hypertensive rats treated chronically with ICI 118,551 (W_mRI and W_fRI, respectively)

	Plasma NA concentration (pmol/ml)	Plasma Ad concentration (pmol/ml)
W_mR	+ 12.8 ± 0.4	+ 23.3 ± 0.7
SHR_m	10.4 ± 0.5	19.4 ± 1.7
W_mRI	9.5 ± 0.9	16.5 ± 2.8
W_fR	+ 12.8 ± 0.6	+ 24.6 ± 0.5
SHR_f	10.5 ± 0.6	20.5 ± 2.1
W_fRI	9.8 ± 0.8	16.7 ± 3.1

Values are given as mean ± s.e of mean
n = 10 animals for each group
+ $p<0.05$ compared to all other groups

6.3iv Effect of ethinyloestradiol (EO) treatment on the plasma NA and Ad levels

Table 6.3 shows the plasma NA and Ad levels of Wistar animals treated chronically with EO (1.5mg/kg sub cutaneously for three weeks) and with the vehicle.

The plasma NA and Ad levels are elevated in the chronic EO treated animals compared to the vehicle treated animals. The plasma Ad levels in the EO treated females tended to be higher than the EO treated males but this effect was not significant.

Table 6.3

Plasma NA and Ad levels in male and female rats chronically treated with ethinyloestradiol (EO_m and EO_f, respectively) and in male and female rats treated with vehicle (W_m and W_f, respectively)

	Plasma NA concentration (pmol/ml)	Plasma Ad concentration (pmol/ml)
EO_m	7.8 ± 0.7	13.6 ± 1.2
W_m	4.9 ± 0.8	9.0 ± 1.4
EO_f	7.6 ± 0.8	15.4 ± 0.8
W_f	4.8 ± 0.6	9.3 ± 0.9

Values are given as mean ± s.e. of mean
n = 10 animals for each group
Plasma NA and Ad levels in EO_m and EO_f significantly greater than in W_m and W_f ($p<0.05$)

6.4 Discussion

The results indicate that the SHR rat generally had an elevated plasma NA and Ad level compared to age matched Wistar rats. Previous reports (McCarty et al, 1978; Popper et al, 1977) indicate that there is no significant difference in the circulating plasma NA and Ad levels between the SH and the Wistar Kyoto (Control) animals. However, McCarty et al (1978) measured basal sympatho-adrenal activity in the undisturbed rat via an indwelling arterial cannula.

Renal hypertension in the Wistar rat tended to elevate the plasma catecholamine levels compared to the control animals and the plasma catecholamine levels were significantly greater than those in the SH animals. ICI 118,551 treatment tended to decrease the increased blood pressure (see Chapter 4) in the renal hypertensive animals; the ICI 118,551 treatment also decreased the plasma catecholamine levels in the renal hypertensive rat.

Chronic EO treatment also elevated the circulating catecholamine plasma levels though not as high as in the SHR or renal hypertensive rats.

It therefore seems that sympatho-adrenal activity plays an important role in the maintenance and persistence of the three forms of hypertension described here.

Drugs used

dl - Propranolol hydrochloride	ICI Pharmaceuticals Ltd
dl - Isoprenaline hydrochloride	ICI Pharmaceuticals Ltd
ICI 118,551 as the hydrochloride	ICI Pharmaceuticals Ltd
Atenolol hydrochloride	ICI Pharmaceuticals Ltd
Angiotensin II (human) Acetate	Sigma Chemicals Ltd
dl - Noradrenaline bitartrate	Sigma Chemicals Ltd
[Sar^1-Ile^8] angiotensin II	Sigma Chemicals Ltd
17α- Ethinyloestradiol	Sigma Chemicals Ltd
Guanethidine (Ismelin)	Giba/Geigy Pharmaceuticals Ltd
Cocaine hydrochloride	Vestric Ltd (Bristol)
Captopril	Squibb Pharmaceuticals Ltd

Atenolol, ICI 118,551, isoprenaline and propranolol were kindly donated by ICI Pharmaceuticals Ltd

CHAPTER 7

DISCUSSION

7.1 Enhanced presynaptic β_2-adrenoreceptor-mediated modulation of vascular adrenergic neurotransmission in Spontaneously Hypertensive rats and New Zealand Hypertensive rats

The results described in this thesis demonstrate that the β-adrenoreceptor agonist, isoprenaline, potentiated the pressor response induced by peri-arterial nerve stimulation of the mesenteric vascular bed and the kidney vasculature without affecting the basal perfusion pressure. This potentiation was enhanced in the preparations from SH and NZH rats compared to those from the Wistar and NZN rats.

In the preparations taken from the normotensive rats, low concentrations of isoprenaline (10^{-11}M to $5x10^{-8}$M) progressively potentiated the effect of PNS; at higher concentrations of isoprenaline the potentiation was replaced by a concentration-dependent inhibition of the pressor response to PNS.

In the preparations from hypertensive rats, a similar shaped curve was obtained; in the mesenteric vascular preparation the potentiation of the pressor response to PNS was significantly greater than that in the respective normotensive rats at all concentrations of isoprenaline. However, in the isolated kidney preparations, the potentiation of the PNS pressor response was significantly greater in the hypertensive rats than in the

respective normotensive rats at the lower isoprenaline concentrations ($<10^{-8}$M), but no inhibition of the pressor response to PNS was observed in the preparations from hypertensive animals at higher concentrations of isoprenaline. This might be attributed to the kidney vasculature being a complex integration of tissues and various humoral and neurohumoral agents interfering with the isoprenaline-induced potentiation of the pressor response to PNS. For example, it has been reported that in the rabbit kidney, prostaglandin E_2 dose-dependently and reversibly inhibited NA overflow resulting from renal nerve stimulation (Frame et al, 1975).

The biosynthesis of prostaglandins has been shown to be ten times greater in the medulla than in the cortex of the kidney, though the cortical synthesis is large enough to be of significance (Larsson and Anggard, 1973). The renal cortex also possesses an abundant amount of the prostaglandin degrading enzyme, prostaglandin dehydrogenase (Anggard, Larsson and Samuelsson, 1971), and it is possible that endogenous cortical prostaglandin which escapes degradation may have access to the cortical nerve endings and affects neurotransmitter release. The effects of all the various humoral and neurohumoral agents found in the kidney vasculature on the isoprenaline induced effects on the pressor responses to PNS and NA are beyond the scope of research presented in this thesis; suffice it to say that isoprenaline caused an enhanced potentiation of the pressor response to PNS in the

preparation from the hypertensive rat compared to the normotensive rats.

Isoprenaline produced a dose-dependent inhibition of the pressor response to infusions of exogenous NA in all preparations. The magnitude of this inhibition was the same in the preparations from the hypertensive and normotensive animals i.e. no difference in the degree of inhibition of the pressor response to exogenous NA between the mesenteric vasculature, or the kidney vasculature, of the hypertensive rats and their respective normotensive rats.

The facilitatory effect of isoprenaline on the pressor response to PNS was markedly reduced by either the non-selective β-adrenoreceptor antagonist, propranolol, or the selective β_2-adrenoreceptor antagonist, ICI 118,551.
The selective β_1-adrenoreceptor antagonist, atenolol, did not inhibit the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations from any of the animals. However, atenolol further potentiated the isoprenaline-induced potentiation of the pressor response to PNS in the preparations from the male SH rat. The isolated kidney preparation from the male SH rat tended to exhibit this further potentiation, but this effect was only statistically significant in the mesenteric vasculature of the male SH rat.

Atenolol, ICI 118,551 and propranolol all reversed the isoprenaline induced inhibition of the pressor response to exogenous NA. This suggests that the isoprenaline induced inhibition of the vasoconstrictor response to exogenous NA could be due to physiological antagonism at postsynaptic receptor sites. It therefore appears that the inhibitory effect of isoprenaline on the pressor response to PNS in the preparations from Wistar and NZN rats probably resulted from the dominant postsynaptic action of isoprenaline (vascular relaxation) masking its presynaptic effects (facilitating NA release, thereby enhancing vasoconstriction). These findings indicate that the dilatation of the mesenteric and kidney vasculature may be mediated through the activation of β_1- and β_2-adrenoreceptors. This could therefore explain the enhancing effect of atenolol on the isoprenaline induced potentiation of the pressor response to PNS in the preparations from male SH rats; the enhancement was probably due to prevention of the β_1-adrenoreceptor mediated physiological antagonism of α-adrenoreceptor-mediated vasoconstriction produced by neurally-released NA.

Previous reports suggest that the sympathetic adrenergic nerve activity may be elevated in the SH rat (Iriuchijima, 1973; Hallbeck and Folkow, 1974; Judy et al, 1976). In the present study, the neurogenic vasoconstrictor response was greater in the SH and NZH rats than in the Wistar and NZN rats. This finding also lends support to the findings

of Kawasaki et al (Kawasaki et al, 1984). The observations in this thesis suggest that enhanced release of NA from vascular adrenergic nerves in response to nerve stimulation may contribute to the enhanced adrenergic vasoconstriction that is suggested to be present in the SH and NZH rat. Since there is also a significant enhancement of the response to NA in the vascular preparations from SH and NZH rats, the degree of the contribution of increased release of NA cannot be assessed in the control response.

Some workers have reported a diminished relaxant response to isoprenaline in the isolated thoracic aorta from the SH rat compared to the Wistar-Kyoto rat (Triner et al, 1975; Cohen and Berkowitz, 1976) and have suggested that the marked difference of facilitatory effect of isoprenaline between the hypertensive and normotensive rats could be due in part to the diminished relaxant effect of isoprenaline in the SH rat. However, the results presented in this thesis show that there is no significant difference between the inhibitory effect of isoprenaline on the pressor response to exogenous NA in the preparations from the respective strains of rats. Therefore it is unlikely that the postsynaptic action of isoprenaline contributes to the enhanced facilitatory effect of the neurogenic pressor response in the preparations from SH and NZH rats compared to Wistar and NZN rats.

Thus far the results indicate that physiological mechanisms facilitating sympathetic neurotransmission in vascular beds are enhanced in the hypertensive rats compared to the respective normotensive rats. That the facilitatory mechanisms are located presynaptically can be inferred from the comparison of the effects of isoprenaline on the pressor responses to PNS and NA infusion; there is a very clear enhancement of the pressor response to PNS by isoprenaline in the SH and NZH rats, but no difference in the dose dependent inhibition of pressor responses to exogenous NA by isoprenaline in any of the preparations from hypertensive and normotensive rats. It can further be concluded that the augmentation of presynaptic activity by isoprenaline is mediated via β_2-adrenoreceptors, since the effect was convincingly abolished by a non-selective β-adrenoreceptor antagonist, propranolol, and a β_2-selective-adrenorececptor antagonist, ICI 118,551, but was unaffected by the presence of a β_1-selective-adrenoreceptor antagonist, atenolol.

7.2. Enhanced presynaptic angiotensin II receptor-mediated modulation of vascular adrenergic neurotransmission in Spontaneously Hypertensive rats and New Zealand Hypertensive rats

The results demonstrate that AII enhanced the pressor responses to exogenous NA and to PNS of the mesenteric and kidney vascular beds without affecting the basal perfusion pressure. The enhancement of the pressor responses to either PNS or NA infusion by AII was effectively antagonised by the competitive AII receptor antagonist, Sar. Sar did not affect the respones to PNS or NA infusion in the absence of AII, therefore suggesting receptor specificity of AII in its potentiating effects.

The degree of the AII induced potentiation of the pressor response to PNS was not different to that observed to infusions of NA in the Wistar and NZN rats; however in the SH and NZH rats the degree of the AII-induced potentiation of the pressor response to PNS was found to be greater than the degree of the AII-induced potentiation of the pressor response to NA infusion.

The degree of AII-induced potentiation of the pressor response to exogenous NA was the same in the hypertensive and normotensive rats, whilst the AII-induced potentiation of the pressor response to PNS was found to be greater in the SH and NZH rats compared to that in the Wistar and NZN rats. This suggests that the facilitatory effect of AII

may be due to increased release of NA in response to sympathetic nerve stimulation, mediated via presynaptic AII receptors. Previous investigators have shown that AII potentiates NA release in response to sympathetic nerve stimulation in some tissues (Starke, 1971; Duckles, 1980).

Some investigators have proposed that AII potentiates vasoconstrictor response by interference with neuronal uptake of NA (Palaic and Khairallah, 1967; Campbell and Jackson, 1979; Jackson and Campbell, 1979). Therefore, the effect of AII was further studied in the presence of cocaine, an inhibitor of neuronal NA uptake. When AII was perfused simultaneously with cocaine the potentiation of the pressor responses to PNS and exogenous NA were higher than those when either of the two drugs were perfused separately. Thus, it is unlikely that inhibition of neuronal uptake by AII accounts for this effect. The facilitatory effect of the response to exogenous NA by AII may be due to nonselective postsynaptic sensitization of this peptide reported by Day and Moore (Day and Moore, 1976).

As previously mentioned, the potentiation of neurogenic pressor response by AII was significantly greater than that of the exogenous NA response in the SH and NZH rats, thus indicating that the increase in neurogenic pressor response is greater than can be accounted for by the postsynaptic sensitizing effect of this octapeptide in the preparations from SH and NZH rats. A greater facilitation

of the response to either PNS or NA by AII in SH and NZH preparations was observed even in the presence of cocaine. These results strongly suggest that facilitatory modulation of neurotransmission, mediated by presynaptic AII receptors, is enhanced in the mesenteric and renal vasculature from SH and NZH rats. It appears that the facilitatory effect of AII on the neurogenic vasoconstrictor response in the preparations from genetically hypertensive animals may be due to increased transmitter release from sympathetic adrenergic nerve endings.

The results thus indicate that in addition to enhanced presynaptic β_2-adrenoreceptor-mediated modulation of sympathetic neurotransmission in the vascular beds of hypertensive animals there is also an enhanced presynaptic AII receptor-mediated modulation of the sympathetic neurotransmission. That the AII receptor mediated facilitatory mechanism is located presynaptically can be inferred from the effects of AII inferred from the effects of AII on the pressor responses to PNS and exogenous NA. This AII-mediated facilitation of adrenergic neurotransmission in vascular beds was enhanced in the SH and NZH rats compared to the Wistar and NZN rats and was equally marked in the male and female SH rats.

Finally, the absence of effect on basal tone of any of the antagonists strongly suggests a lack of endogenous agonists mediating any significant degeree of tone in these isolated preparations.

7.3 Interaction between presynaptic AII receptor and presynaptic β_2-adrenoreceptor in the modulation of adrenergic neurotransmission in the Spontaneously Hypertensive rat and New Zealand Hypertensive rat

The results demonstrate that presynaptic mechanisms that facilitate vascular sympathetic neurotransmission involve both β_2-adrenoreceptors and AII receptors. The results also suggest how these two mechanisms may interact with one another.

The isoprenaline-induced enhancement of the pressor response to PNS was effectively inhibited by Sar in preparations from all the strains of rats. This inhibition was more marked in the SH and NZH rats than in the Wistar and NZN rats (since the isoprenaline-induced potentiation of the pressor response to PNS was markedly enhanced in the hypertensive rats compared to the normotensive rats). The angiotensin II receptor antagonist did not affect the isoprenaline-induced inhibition of the pressor response to NA infusion in the preparations from any of the rats. This finding led to the possibility that the facilitatory effect of presynaptic β_2-adrenoreceptors on vascular adrenergic neurotransmission was linked to the presynaptic AII receptor in the vascular beds of the rats. Indeed, all the components necessary for AII synthesis are present in the wall of blood vessels (Desjardins-Giasson et al, 1981; Dengler, 1956; Dzau, 1984; Ljung et al, 1981; Velletri and

Bean 1982). Captopril, an angiotensin converting enzyme inhibitor , effectively inhibited the isoprenaline-induced potentiation of the pressor response to PNS in preparations from all types of rat without affecting the isoprenaline-induced inhibition of the pressor response to exogenous NA. Once again, the inhibition of the isoprenaline-induced potentiation of the PNS pressor response was more marked in the SH and NZH rats than in the Wistar and NZN rats.

ICI 118,551 did not affect the AII-induced potentiation of the pressor responses to PNS and NA infusion in any of the preparations from any of the animals.

There have been reports that captopril causes non-specific depression of the vascular response to NA in isolated blood vessels (Okuno et al, 1979; Kikta and Fregly, 1982), however this did not seem to be the case in this study. In the previously reported studies the concentrations of captopril employed were in the order of 10^{-5}M to 10^{-4}M. ICI 118,551 did not affect what is assumed to be the postsynaptically-mediated potentiation by AII of the pressor response to NA.

It appears then that locally generated AII, resulting from presynaptic β_2-adrenoreceptor stimulation contributes to the greater enhancement of neurogenic response by isoprenaline in the SH and NZH rats compared to the normotensive Wistar and NZN rats.

7.4. Goldblatt, two-kidney, one-clip renal hypertension: Involvement of facilitatory presynaptic receptors

The results described in this thesis show that the two-kidney, one-clip procedure was effective in inducing hypertension in the Wistar rat. This procedure also caused an enhanced vasoconstrictor response to PNS and exogenous NA in the isolated mesenteric vasculature. This increased responsiveness to PNS and to exogenous NA could indicate an increased vascular tone in the renal hypertensive rats and might further suggest that this increased tone could contribute to the hypertension in this experimental model.

Since enhancement of presynaptic β_2-adrenoreceptor-mediated and presynaptic angiotensin II-receptor-mediated facilitation of adrenergic neurotransmission in the vascular beds of hypertensive rats has been shown previously, the possibility of these two presynaptic facilitatory mechanisms being enhanced in the Goldblatt, two-kidney, one-clip renal hypertensive rat was investigated. Indeed, the results presented in this thesis show that the physiological mechanisms facilitating sympathetic neurotransmission in the mesenteric vascular bed are enhanced in the renal hypertensive rats compared to the normotensive Wistar rats. That the facilitatory mechanisms are located presynaptically can be deduced from the comparison of the effects of isoprenaline and angiotensin II on the pressor response to PNS and NA

infusion. It can be further concluded that the augmentation of presynaptic activity by isoprenaline is mediated via β_2-adrenoreceptors and that of angiotensin II via pre-synaptic angiotensin II receptors.

It follows from these deductions that if the concept of presynaptic β_2-adrenoreceptor-mediated facilitation of adrenergic neurotransmission is accepted then antagonism of the presynaptic β_2-adrenoreceptor would attenuate, at least to some degree, this facilitatory mechanism. In the isolated mesenteric vascular preparation from renal hypertensive rats treated chronically with ICI 118,551 the isoprenaline-induced potentiation of the pressor response to PNS was completely abolished compared to that of the untreated renal hypertensive rat. Chronic treatment with ICI 118,551 did not affect the isoprenaline-induced inhibition of the pressor response to NA infusion compared to that in the untreated renal hypertensive rats. Indeed, the progression of the hypertension in the Goldblatt, two-kidney, one-clip renal hypertensive rats was significantly attenuated by chronic administration of ICI 118,551. However, chronic treatment of the renal hypertensive rats with ICI 118,551 did not completely abolish the rise in systolic blood pressure. This inability of ICI 118,551 to completely arrest the rise in systolic blood pressure in the renal hypertensive rats could be attributed to other mechanisms contributing to the hypertension rather than the sole involvement of the presynaptic β_2-adrenoreceptor. One of these other mechanisms contributing to the

hypertension could well be the renin-angiotensin system. In the preparations from renal hypertensive rats, and even from renal hypertensive rats treated chronically with ICI 118,551, the angiotensin II-induced potentiation of the pressor responses to PNS and exogenous NA were significantly enhanced compared to the normotensive control Wistar rats.

The pressor response to exogenous NA in the preparations from renal hypertensive rats treated chronically with ICI 118,551 did not differ from that in the untreated renal hypertensive rats. However, the pressor response to PNS in the preparations from renal hypertensive rats treated chronically with ICI 118,551 was significantly lower than that in the untreated renal hypertensive rats. This indicates that chronic treatment with ICI 118,551 affected neural release of NA since postsynaptic responses to NA infusion were not different in the preparations from each group.

One of the possible ways that ICI 118,551 could attenuate the release of neural NA is by blockade of the facilitatory presynaptic β_2-adrenoreceptor. Chronic treatment with ICI 118,551 probably antagonizes the presynaptic β_2-adrenoreceptor during the progression of the hypertension in the two-kidney, one-clip renal hypertensive rats. However, from the studies in the isolated perfused mesenteric vascular preparations it seems that chronic treatment of the renal hypertensive

rats with ICI 118,551 completely abolished the presynaptic B_2-adrenoreceptor-mediated facilitation of adrenergic neurotransmission in the preparations. This could be because the chronic treatment with ICI 118,551 had a direct effect on the presynaptic B_2-adrenoreceptors by "deactivating" them; hence perfusion of B-adrenoreceptor agonists in the tissue from rats chronically treated with ICI 118,551 would not have any affect on the pressor response to PNS.

Alternatively, chronic treatment with ICI 118,551 could directly attenuate the turnover of NA in the postganglionic nerve endings. However in the work carried out by Bilski et al (1983), ICI 118,551 did not affect the heart rate in beagle dogs thereby indicating that ICI 118,551 did not affect NA turnover.

It has been reported that ICI 118,551 has membrane-stabilizing potency similar to that of propranolol (Bilski et al, 1983), and that this membrane-stabilizing effect could attenuate the PNS-induced release of NA from the nerve endings; however, if the membrane-stabilizing effect of ICI 118,551 was responsible for the decreased pressor response to PNS then the vasoconstrictor response to PNS in the preparations from normotensive control rats would be attenuated when ICI 118,551 was perfused through them compared to the PNS response when physiological saline (Krebs solution) was perfused through them. Therefore this reason too seems improbable. It therefore seems then

that ICI 118,551 reduces the pressor response to PNS via antagonism of presynaptic β_2-adrenoreceptors in the mesenteric vasculature and that the decreased vasoconstriction leads to a decreased peripheral resistance and therefore to a decreased blood pressure. It remains to be seen whether this effect of ICI 118, 551 is true for other vascular preparations besides the mesenteric vascular bed.

It has been demonstrated that plasma renin activity is elevated in the two-kidney, one-clip model of hypertension (Horovitz, 1981; Sweet et al, 1984; Laffan et al, 1978; Iso et al, 1981) and this would imply that circulating plasma levels of angiotensin II would be elevated in the renal hypertensive models compared to that in normotensive animals. Due to the nonselective postsynaptic sensitization to NA by AII (Day and Moore, 1976), which has also been demonstrated in this thesis, the increased vasoconstrictor effect of NA on postsynaptic tissue would contribute to the increased vascular tone and therefore the increased blood pressure in the renal hypertensive rats compared to normotensive control rats. Coupled to this, the increased concentration of circulating angiotensin II in the renal hypertensive rats could activate presynaptic AII receptors thereby releasing increased amounts of NA into the general circulation in response to nerve impulses and therefore contributing to the overall increase in vascular resistance and increasing blood pressure. Indeed, the basal perfusion pressure of

the preparations from renal hypertensive rats has been demonstrated to be higher than that in the normotensive rats. The results show that in the preparations from renal hypertensive rats treated chronically with ICI 118,551 and from untreated renal hypertensive rats the presynaptic angiotensin II-receptor-mediated facilitation of adrenergic neurotransmission is enhanced compared to that in normotensive control rats.

The plasma levels of Ad and NA in the renal hypertensive rats were significantly higher than those in the renal hypertensive rats treated chronically with ICI 118,551 and both these types of rats had significantly elevated levels of plasma Ad and NA compared to the Wistar rats. The results from the experiments involving preparations from renal hypertensive rats indicate that the sympatho-adrenal medullary activity may be increased and that the adrenal medulla may contribute to the progression of the hypertension in this model.

However, Finch and Leach (1970) have shown that experimental hypertension in the rat is independent of the adrenal medulla provided an adequate salt intake is maintained. However, the results in this thesis indicate that there may be some contribution of the adrenal medulla in the maintenance of the hypertension in the two-kidney, one-clip model of renal hypertension since plasma Ad levels in the renal hypertensive rats were elevated compared to control Wistar rats; also chronic treatment

with ICI 118,551 may have antagonised the action of adrenaline at the presynaptic β_2-adrenoreceptors thereby attenuating the progression of the blood pressure in this model of renal hypertension. There has been evidence that there is an elevation of plasma renin activity and elevated concentration of renin in the arterial walls of experimental renal hypertension (Garst et al, 1979). Furthermore, Garst et al (1979) have shown that as the renal hypertension becomes chronic, the concentration of renin in the arterial wall remains elevated as the plasma renin activity is reduced to normal or sub-normal levels. Thus, the increased arterial wall renin is associated with the elevated blood pressure in the acute stage of renal hypertension, and increased renin content may well continue to maintain the hypertension in the chronic stage despite the fall in plasma renin at this stage (Garst et al, 1979). These results lend supoport to the findings of this thesis that the presynaptic angiotensin II receptors are enhanced in the isolated mesenteric vascular preparations from renal hypertensive rats and this indicates an enhanced vascular renin-angiotensin system in the mesenteric vascular beds of renal hypertensive rats.

Thus the results indicate that in the Goldblatt, two-kidney, one-clip renal hypertensive rat, the development and maintenance of the hypertension is partly dependent on the presynaptic β_2-adrenoreceptor-mediated facilitation of neurogenic pressor response and probably more dependent on a local renin-angiotensin system present in the arterial

walls of the vasculature, the effects of which are mediated via presynaptic angiotensin II-receptors which facilitate adrenergic neurotransmission in the vasculature.

7.5 Oestrogen-induced hypertension: Involvement of presynaptic β_2-adrenoreceptors

The results demonstrate that there is a decrease of body weight of rats treated chronically with EO compared to the non-treated rats. It is suggested that the decrease in body weight after chronic EO adminstration may be due to either (i) anoretic and/or (ii) antiandrogenic actions of EO (Meites, 1949; Glesser, 1954). The decrease in body weight was significantly greater in the male rats treated with EO than in the female rats similary treated. The blood pressure of rats chronically treated with EO was elevated compared to the normotensive untreated rats. This elevation was significantly higher in the female rats than in the male rats, confirming the findings of Bhatt et al (Bhatt and Gulati, 1986). This sex-related difference could be due to the presence of androgenic hormones in the male rats which might tend to inhibit the peripheral vascular actions of catecholamines and neurohypophyseal hormones (Altura, 1973; Greenberg et al, 1973).

In addition, it has been reported that mesenteric arterioles of female rats are more sensitive than those of males to vasoconstrictor action of catecholamines and neurohypophyseal hormones (Altura, 1975). However, in this study the results show that there was no significant difference between the male and female rats treated with EO in their responses to PNS and NA infusions in the mesenteric and renal vasculature.

The pressor responses to PNS and NA infusion in the preparations from rats treated chronically with EO were enhanced compared to the untreated rats. The enhanced responsiveness to NA infusion in the EO-treated rats could be explained by alteration in the structural component of the resistence vessels (McGregor and Smirk, 1970; Folkow and Hallbeck, 1977) and an increase in the number of α-adrenoreceptors. After oestrogen treatment increase in the number of α-adrenoreceptors has been demonstrated in extravascular smooth muscles, without change in their affinity (William and Lefkowitz, 1977; Rand et al, 1977). However, Colucci et al (1982) using a radioligand technique, proposed that oestrogen-induced increase in sensitivity to catecholamines of the mesenteric vascular bed may be partly mediated through an increase in α-adrenoreceptor affinity. In the present study no attempt was made to study affinity of the receptors in vascular bed to NA or the increase of α-adrenoreceptors.

The role of the adrenal medulla in rats made hypertensive with androgen treatment (DeChamplain, 1977) and with oestrogen treatment (Bhatt et al, 1986) has been implicated previously. Indeed, significant increases in dopamine-β-hydroxylase (DBH) activity in the adrenal glands of EO-treated animals have been reported (Bhatt et al, 1986). It seems possible that the increase in DBH activity in adrenal medulla after chronic EO treatment may increase the synthesis of catecholamines and subsequently lead to release of more catecholamines in the general

circulation producing elevation in blood pressure. Increases in NA levels in hypothalamic regions and in the adrenal medulla have been previously reported (Lew, 1982) after higher doses of mestranol, whereas decreases have been described in young rats receiving lower doses of mestranol (Lew, 1975; 1978).

In the work described by Bhatt and Gulati (1986), the increase in weight of the adrenal glands from EO-treated rats compared to untreated rats could be attributed to the hypertrophic appearance of the gland which in turn could be caused by increased DBH activity in the adrenal glands. The results presented in this thesis also lend support to these findings in that I found that the weights of adrenal glands from rats treated chronically with EO are greater than those from untreated normotensive rats.

In addition to the above possibility for the increased blood pressure and increased response to NA infusions in rats treated chronically with EO, another suggestion may be advanced and that is the involvement of the presynaptic β_2-adrenoreceptor and the presynaptic angiotensin II receptor which have been previously identified in the SH and NZH rats.

The results described in Chapter 5 show an enhanced isoprenaline-induced potentiation of the pressor response to PNS in the preparations from rats treated chronically with EO compared to those from untreated normotensive

rats. There were no differences in this enhancement between the equivalent preparations from male and female rats. Isoprenaline caused an inhibition of the pressor reponse to NA infusion in all the preparations. The degree of this inhibition was the same in the preparations from the oestrogen-induced hypertensive rats and normotensive rats, indicating that the mechanisms of vasodilatation in the vascular beds from the oestrogen-induced hypertensive rats and normotensive rats are not dissimilar. Isoprenaline alone had no effect on the basal perfusion pressure of any of the preparations.

The facilitatory effect of isoprenaline on the pressor response to PNS was markedly reduced by ICI 118,551 in all the preparations. Atenolol did not inhibit the isoprenaline induced potentiation of the pressor response to PNS in any of the preparations. Neither ICI 118,551 nor atenolol had any effect on the basal perfusion pressure or the pressor response to PNS in any of the preparations. However, atenolol and ICI 118,551 effectively reversed the isoprenaline induced inhibition of the pressor response to NA infusion in preparations from all of the animals.

AII caused a potentiation of the pressor response to PNS in all the preparations. This potentiation was of a similar magnitude in the oestrogen-induced hypertensive rats and normotensive rats. AII also caused a potentiation of the pressor response to exogenous NA in

all the preparations. Once again, this potentiation was of a similar magnitude in the oestrogen-induced hypertensive rats and the normotensive rats. The results from experiments involving AII therefore show that in the vasculature from animals chronically treated with EO the presynaptic AII receptor mediated modulation of adrenergic neurotransmission is not dissimilar to that in the normotensive rats i.e. it is not enhanced as found in the SH, NZH and renal hypertensive rats.

Neither captopril nor Sar had any effect on the basal perfusion pressure or the pressor responses to PNS and exogenous NA in any of the preparations, however they both inhibited the isoprenaline-induced potentiation of the pressor response to PNS in all the preparations. Neither captopril nor Sar had any effect on the isoprenaline induced inhibition of the pressor response to exogenous NA in any of the preparations.

The results indicate that local generation of AII is required for the enhancement of adrenergic neurotransmission in the vasculature of EO-treated rats via presynaptic β_2-adrenoreceptors since both captopril and Sar effectively inhibited the isoprenaline-induced potentiation of the PNS pressor response in those tissues. The sequence of events in the facilitation of adrenergic neurotransmission in the vasculature from oestrogen-induced hypertensive rats is probably the activiation of presynaptic β_2-adrenoreceptors which could activate a

vascular renin-angiotensin system to generate AII locally. AII produced this way then causes a modest enhancement of the pressor response to PNS.

It therefore appears that the presynaptic β_2-adrenoreceptor facilitatory mechanism is enhanced in the vasculature from oestrogen-induced hypertensive rats compared to normotensive rats. This enhanced facilitatory effect is qualitatively and quantitatively similar in the preparations from male and female rats chronically treated with EO.

7.6 Plasma adrenaline and noradrenaline levels

The method of collecting blood samples from rats for plasma catecholamine analysis affects the determination of the catecholamine concentration in the blood samples.

McCarty et al (1978) have previously shown that the plasma NA and Ad levels in the SH and Wistar-Kyoto rats vary when the blood samples are collected in the undisturbed, mildly disturbed and shocked rats. Decapitation of rats in order to collect blood samples increases the plasma Ad and NA levels (Popper et al, 1977).

The method used for the collection of the blood samples in this thesis involved placing the animal in a bell-jar and inducing anaesthesia with ether. This procedure tended to cause a degree of stress in the animal, however, the procedure was the same for all the rats used in this study.

The results show that the plasma Ad and NA levels in the SH rats are elevated compared to those from Wistar rats. The elevation is significant in the SH rats from the age of three weeks onwards. No difference in the plasma catecholamine levels between the males and females of each strain of rats was observed. A rapid increase in plasma NA and Ad levels in the SH rats was observed between the ages of three to twelve weeks. An elevated plasma NA level in SH rats has been noted only during the

prehypertensive state (Yamori, 1974; Grobecker et al, 1975) and not during the maintenance stage (Yamori, 1974). On the other hand , Vlachakis et al (1980) reported an increased plasma concentration of normetanephrine in SH rats. Normetanephrine is an extraneuronal metabolic product of NA and provides a useful index of sympathetic function (Vlachakis et al, 1980).

Plasma NA and Ad levels in the two-kidney, one-clip renal hypertensive rats were found to be significantly elevated compared to those from age-and-sex matched SH and Wistar rats. The plasma catecholamine levels in the renal hypertensive rats chronically treated with ICI 118,551 were not different to those from age-and-sex-matched SH rats but significantly higher and lower than those from age-and-sex-matched Wistar and untreated renal hypertensive rats respectively.

The plasma NA and Ad levels in the Wistar rats chronically treated with EO were elevated compared to those from the normotensive Wistar rats. Howevever, this elevation was significantly less than that found in the SH rats. The hypertrophic nature and increase in weight of the adrenal glands observed could be related to the increase in dopamine-β-hydroxylase activity in the gland previously reported (Bhatt et al, 1986).

There is an indication that plasma levels of Ad in the female rats treated chronically with EO are slightly

elevated compared to those from male rats similarly treated. This could probably explain the higher systolic blood pressure in the female EO treated rats than in the male EO treated rats.

It therefore seems that sympatho-adrenal activity plays an important role in the maintenance and persistence of the three forms of hypertension described in this thesis. Indeed, an increase in the SH rat of the adrenal medullary activity of the rate-limiting enzyme for catecholamine biosynthesis, tyrosine hydroxylase, has been reported in utero (Teitelman et al, 1981) and in the adult SH rat (Grobecker et al, 1982). The Ad and NA content of the adrenal medulla of the SH rat has also been reported (Worcial et al, 1977) to be elevated compared to the control normotensive rats.

7.7 General Discussion

The results described in this thesis indicate that presynaptic β_2-adrenoreceptor-mediated facilitation of the neurogenic pressor response in vascular tissues of hypertensive rats is enhanced compared to that of normotensive control rats. This facilitatory mechanism is qualitatively and quantitatively similar in male and female rats irrespective of whether the hypertension was experimentally induced or genetically inherent. In all the hypertensive models, except that of the oestrogen-induced hypertension, there also exists an enhanced presynaptic AII-receptor-mediated facilitation of the neurogenic pressor response in the vasculature.

This facilitatory mechanism is qualitatively and quantitatively similar in male and female SH, NZH and renal hypertensive rats. The results obtained in this thesis imply that an enhanced local renin-angiotensin system exists in the arterial walls of SH, NZH and renal hypertensive animals and that this system, coupled with that of the presynaptic β_2-adrenoreceptor, contributes to the development and/or maintenance of hypertension.

Previous reports have indicated that the renin-angiotensin pressor system does not participate in the maintenance of hypertension in the SH rats (Sokabe, 1965; Koletsky et al, 1970) and that the hypertensive state is maintained by an increase in neurogenic vasoconstrictor influences (Nosaka

et al, 1972; Okamoto et al, 1967; Yamori et al, 1969) which could theoretically be associated with an increase in the turnover of catecholamines (Molinoff et al, 1971). However, a number of reports indicate a decrease in catecholamine turnover in peripheral tissue from SH rats (Nakamura et al, 1971; Yamori et al, 1972). Other workers have suggested that structural changes of blood vessels may be involved in the maintenance of the hypertensive state in the SH rat (Folkow et al, 1972; Mulvany, 1984). According to this suggestion, increased media/lumen ratio causes a high basal vascular resistance and changes in the responsiveness to vasoconstrictor stimuli.

SH rats are of interest to experimentalists and clinicians as they provide close facsimiles of essential hypertension in humans (Grollman, 1972), therefore it is desirable to define more clearly what causes and maintains the hypertension in the SH rat.

The components of two facilitatory mechanisms mentioned above are therefore present in the normotensive rats but the mechanisms are not "activated" or enhanced as in the SH and NZH rats. Results from the experiments involving the two-kidney, one-clip renal hypertensive rats indicate that the occlusion of the renal artery causes an increase in the blood pressure of the animals, possibly due to an increased sympatho adrenal activty (Asaad et al, 1982) and an increased plasma and arterial wall renin activity (Garst et al, 1979). Indeed in the preparations from

renal hypertensive rats the presynaptic β_2-adrenoreceptor-mediated facilitation of neurogenic pressor response was enhanced compared to that in normotensive control animals. In addition, presynaptic AII-receptor-mediated facilitation of the neurogenic pressor response also appeared to be enhanced in this model of hypertension. Chronic treatment of renal hypertensive rats with ICI 118,551 significantly reduced the development of the hypertension and antagonised the isoprenaline-induced potentiation of the PNS pressor response in the isolated mesenteric vasculature, thus indicating that the presynaptic β_2-adrenoreceptor-mediated facilitation of the neurogenic pressor response partly contributed to the maintenance of the hypertension in the renal hypertensive rat. In support of this, plasma Ad levels in the renal hypertensive rats were found to be elevated compared to those in the normotensive Wistar rats. The plasma Ad and NA levels in the renal hypertensive rat treated chronically with ICI 118,551 were similar to those in the SH rats and the levels of the catecholamines in these two models were lower than those in the untreated renal hypertensive rat.

The results from the experiments involving preparations from rats treated chronically with EO show that presynaptic β_2-adrenoreceptor-mediated facilitation of adrenergic neurotransmission is enhanced in these preparations compared to those from normotensive Wistar rats. The plasma levels of Ad, and NA also, were elevated

in the EO-treated rats compared to those in the normotensive rats. No enhancement of the facilitatory presynaptic AII-receptor-mediated activity was observed in the preparations from EO-treated rats compared to those from normotensive rats. The adrenal glands from EO-treated rats were heavier and appeared to be hypertrophic in nature compared to those from normotensive rats. Thus this indicates that sympatho-adrenal mediated activity was the main contributor to this form of hypertension.

The possibility that the chronic treatment with EO did not elevate the systolic blood pressure of the rats to as a high degree as that caused by renal artery occlusion could be attributed to an enhanced presynaptic alpha -adrenoreceptor-mediated inhibition of the pressor response to PNS. There have been reports of oestrogens increasing the affinity of α-adrenoreceptors in blood vessels of rats (Colucci et al, 1982) and in the uterus of rabbits (Hoffman et al, 1982). Thus it could be that in the rats treated chronically with EO the oestrogen could have increased the sensitivity of the presynaptic alpha -adrenoreceptor thereby attenuating the increase in systolic blood pressure thought to be mediated via facilitatory presynaptic β_2-adrenoreceptors.
However, though the increased contribution of the sympatho-adrenal activity is implied in this form of hypertension, both captopril and Sar effectively inhibited the isoprenaline-induced potentiation of the pressor response to PNS in the preparations from EO-treated rats.

Thus the results described in this thesis show that the effects mediated via facilitatory presynaptic β_2-adrenoreceptors are linked to a local renin-angiotensin system and that it is AII, or an AII-like substance, that finally facilitates adrenergic neurotransmission in vascular preparations from hypertensive rats. The evidence from the two experimental models of hypertension could give some insight as to the development and/or maintenance of hypertension in the genetically hypertensive rats. The facilitatory effects of presynaptic β_2-adrenoreceptors are invariably mediated finally via angiotensin II, or AII-like product, in all of the hypertensive models in this study. That the presynaptic AII-receptor-mediated facilitatory mechanism is enhanced in all the hypertensive models described in this study (except that of rats made hypertensive by chronic treatment with EO) indicates that the two facilitatory mechanisms are interdependant. Though these two facilitatory mechanisms are inter-related, either of them could be independently activated to facilitate adrenergic neurotransmission in vascular tissue.

It seems that experimentally-induced hypertension causes increased amounts of catecholamines, and probably AII, in the plasma. The surge of these chemicals could alter the affinity and/or the number of presynaptic β_2-adrenoreceptors and presynaptic AII receptors.

Blaustein (Blaustein, 1985) suggests that a sodium-pump inhibitor, natriuretic hormone, promotes natriuresis whilst at the same time increasing peripheral vascular resistance through a direct action on vascular smooth muscle. A functional (genetic or acquired) defect in renal excretion (undetectable by standard renal function tests) can, in the presence of an excessive salt load, lead to the development of hypertension. The initial tendency toward salt and water retention and extra cellular fluid volume expansion is compensated by the secretion of a natriuretic hormone that promotes Na excretion by inhibiting Na pumps in renal tubule cells. This hormone could also inhibit the Na pump in other types of cells, including vascular smooth muscle cells. Then, because the vascular smooth muscle cells contain Na/Ca exchange transport mechanisms in their plasma membranes, more Ca than normal would be delivered to these cells. This could therefore cause the increased contractility and reactivity associated with increased vascular tone and peripheral vascular resistance.

This inhibition of the Na pump could amplify the effects of adrenergic agents, and other humoral agents, on the vascular smooth muscle cells thereby enhancing adrenergic neurotransmission in the vascular beds of the preparations. This thus indicates that the sensitivity rather than possibly the increase in number of presynaptic facilitatory mechanisms may be increased in the hypertensive rat than in the normotensive rat.

The experimental hypertension could, for instance, release one or more substances which in turn sensitizes the presynaptic facilitatory mechanisms thereby causing the mechanism to actively maintain the hypertensive state. Work done previously (McMurtry et al, 1981) has shown that an animal genetically programmed to be normotensive can become hypertensive if it is nursed by a hypertensive mother. Some factor, probably a cerebrohypothalamic neurotransmitter, may be transmitted through the milk, triggering the pathogenesis of progressively increasing blood pressure. It appears that this factor is specific in genetic transcription since not all genetically normotensive weanlings nursed by hypertensive dams responded by developing hypertension (McMurtry et al, 1981).

This factor, a hypertensinogen, that will induce high blood pressure de novo, has been sought in hypertensive humans (Sen et al, 1977). Although this factor causes increased aldosterone-secretion, sodium retention, blood volume expansion, and sustained hypertension - all reduced by adrenalectomy - it is not adrenocorticotrophic hormone, renin, or angiotensin (McMurtry et al, 1981; Sen, 1977). It has been shown (Sen et al, 1977) that a compound has been isolated from normal human urine and it contains a protein fraction that interacts with the adrenal cortex and induces hypertension in rats that is characterized by volume expansion, suppressed renin activity, and marked increases in plasma aldosterone. It is probable that such

a compound may be transmitted from the mothers milk and trigger the pathogenesis of hypertension in the rat.

In addition to the possible involvement of cerebropeptides in the triggering of hypertension, a second messenger may be involved. This second messenger could come from the adrenal glands since adrenalectomised rats reduce the effects of the hypertensinogen (probably a cerebrohypothalamic neuropeptide). A possible second messenger could be the polypeptide, natriuretic hormone, the role of which has been discussed above.

It may well be that chronic treatment with EO and ICI 118,551 has central actions in the rat, and that these two chemicals alter the state of the hypertension. For example, it has been shown that long term administration of EO caused a significant increase in pituitary and adrenal gland size in the rat (Leonara and Crane, 1970) and that women taking oral contraceptives have an increased serum dopamine-β-hydroxylase activity (Rockson et al, 1975). Propranolol, a non-selective β-adrenoreceptor antagonist, has been reported to have a central hypotensive effect (Alexander et al, 1975; Garvey et al, 1975; Day et al, 1973; Reid et al, 1974).

Conversely, it follows from these examples that catecholamines, AII and/or other chemicals (probably cerebropeptide neurotransmitters) may have a central role in the development and/or maintenance of the hypertension.

Therefore, the increased total vascular resistance resulting from enhanced sympathetic adrenergic nerve tone, thought to be the cause of the increased blood pressure in the SH rat (Iriuchima, 1973; Cheng et al, 1980), could be the prime candidate in the role of the development and maintenance of the hypertension in the genetically hypertensive rat. Indeed, Antonaccio (1985) suggests that angiotensin I is converted to AII in the intimal layer of an artery and that the locally generated AII would facilitate sympathetic neurotransmission in the medial layer of the artery. This suggestion is supported by the findings of Mulvany (1984), that the media/lumen ratio of resistance vessels in the SH rat is increased. This could imply that a greater conversion of angiotensinogen to angiotensin II takes place in the resistance vessels. Thus the locally generated AII could then facilitate sympathetic neurotransmission in the medial layer of the blood vessel.

Indeed, work done previously indicates that angiotensin-converting-enzyme (ACE) inhibitors are effect in lowering blood pressure (Nakata et al, 1987; Okuno et al, 1979; Kikta et al, 1982) and because of this the ACE inhibitors are gaining popularity in their effectiveness as antihypertensive agents.

It therefore seems that the development and maintenance of the hypertension in the genetic model largely results from an increased presynaptic β_2-adrenoreceptor mediated

activation of the vascular renin-angiotensin system thereby releasing locally generated AII which in turn facilitates adrenergic neurotransmission in the vasculature

It remains to be seen whether the facilitatory systems and other features contributing to the hypertension in genetic models are genetically pre-determined, or whether there are one or more substances that are released (genetically pre-determined) and which alter the normal functioning of the haemodynamics in the rat and lead to the features that contribute to the hypertension.

7.8 Clinical implications

Stimulation of presynaptic β_2-adrenoreceptors facilitates the release of NA from sympathetic nerve endings. The hypertensive action of adrenaline infusion in the rat (Majewski et al, 1981b) may be explained by such a mechanism. It has been shown in man that low-dose infusion of Ad increases plasma NA (Nezu et al, 1983; Musgrave et al, 1984; Vincent et al, 1983) and that it amplifies sympathetic pressor effects (Vincent et al, 1983). Conversely, by blocking these presynaptic β_2-adrenoreceptors, blood pressure could be lowered.

Antagonism of the presynaptic B-adrenoreceptor could also reduce the amount of locally-generated AII being formed and therefore lower the blood pressure. Indeed, ICI 118, 551 does lower blood pressure in man (Vincent et al, 1985). The effect of ICI 118,551 could also have been mediated by the antagonism of the presynaptic B_2-adrenoreceptors in the juxtaglomerular apparatus thereby reducing plasma renin (Vincent et al, 1985). However, it is important to note that the blood pressure fall in hypertensive man due to administration of propranolol or ICI 118,551 does not correlate with renin suppression (Vincent et al, 1985). It is probable that the antihypertensive effect of ICI 118,551 correlates with arterial ACE activity.

The involvement of tissue ACE suggests the indication of using ACE inhibitors in the management of essential hypertension. Work done in the SH rat by Nakata et al, (1987) show that a good positive correlation exists between basal blood pressure and ACE activity in the aorta. Nakata et al, (1987) suggest further that the antihypertensive action of SA446, an ACE inhibitor, may be due to inhibition of arterial ACE activity in addition to inhibition of plasma and kidney ACE activity.

Therefore, selective β_2-adrenoreceptor antagonists and ACE inhibitors with a high selectivity for tissue ACE activity probably have important roles in the management of essential hypertension in humans.

7.9 Suggestions for possible future work

The work described in this thesis shows the presence of presynaptic receptor-mediated mechanisms facilitating adrenergic neurotransmission in the isolated mesenteric vascular preparation and in the isolated kidney preparation. More research should be carried out, along the same lines as in this study, in other isolated vascular preparations, for example, the rat caudal artery. This would be done in order to see if the findings of this thesis are true for other isolated vascular preparations. In addition to this, other suggestions are:-

a) Effect of chronic treatment with ACE inhibitors in hypertensive rats (genetic and experimental models). The effect of the chronic treatment on presynaptic receptor-mediated facilitation of adrenergic neurotransmission in isolated vascular preparations could be looked at.

b) Effect of chronic treatment with selective β_2-adrenoreceptor antagonists in hypertensive rats (genetic and experimental models). The effect of the chronic treatment on the facilitatory presynaptic mechanism in isolated vascular preparations could also be looked at.

c) Since a central mechanism is also implicated in the development and maintenance of hypertension, the central role of adrenaline and angiotensin II could be looked at.

d) Effect of hypophysectomy on the progression of the blood pressure in genetic models of hypertension. The effect of hypophysectomy on the enhanced presynaptic receptor-mediated facilitation of adrenergic neurotransmission in isolated vascular tissue could be investigated. This could give insight to the possibilities of cerebrohypothalmic neurotransmitters being involved in the induction and/or development of hypertension.

e) Following on from "d" the possibility of linking cerebropeptide-mediated effects with the functioning of the adrenal glands in regard to the development of hypertension. The possibility of a second messenger, probably from the adrenal glands, being involved in the development of the hypertension.

REFERENCES

Ablad, B.L., Johansson, B., Waldeck,B. : Inhibitory effect of propranolol on the vasoconstrictor response to sympathetic nerve stimulation. J. Pharm. Pharmacol 1970; 22, 627-628.

Adler-Graschinsky,E., Langer,S.Z. : Possible role of a β-adrenoreceptor in the regulation of noradrenaline release by nerve stimulation through a positive feed-back mechanism. Br. J. Pharmacol 1975; 53, 43-50.

Alexander, R.W., Davis, J.N., Lefkowitz, R.J. : Direct identification and characterization of beta-adrenergic receptors in rat brain. Nature 1975; 258, 437-439.

Altura, B.M. : Selective microvascular constrictor actions of some neurohypophyseal peptides. Eur. J. Pharmacol. 1973; 24, 49-60.

Anggard, E., Larsson, C. and Samuelsson, B. : The distribution of 15-hydroxyprostaglandin dehydrogenase and prostaglandin - Δ^{13} - reductase in tissue of the swine. Acta physiol. Scand. 1971; 81, 396-404.

Anton, A.H. and Sayre, D.F. : A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther, 1962; 138, 360-375.

Antonaccio, M.J. : The role of the vascular renin-angiotensin system on sympathetic function and vascular responsiveness. Vascular neuroeffector mechanisms. 1975; 361-365. Edited by: Bevan, J.A., Godfraind, T., Maxwell, R.W., Stocklet, J.C., Worcel, M.

Assad, M.M. and Antonaccio, M.J. : Vascular wall renin in spontaneously hypertensive rats. Hypertension. 1982; 4, 487-493.

Baudoin, M., Meyer, P. and Worcel, W. : Specific binding of ^{3}H-angiotensin II in rabbit aorta. Biochemical and Biophysical Research Communications 1971; 42, 434-440.

Bhatt, J.D. and Gulatti, O.D. : The role of the sympathetic nervous system in oestrogen-induced hypertension in rats. Br.J. Pharmacol. 1986; 89, 685-691.

Bilski, A.J., Halliday, S.E., Fitzgerald, J.D. and Wale, J.L. : The pharmacology of a β_2-selective adrenoreceptor antagonist (ICI 118,551). Journal of Cardiovascular Pharmacology. 1983; 5, 430-437.

Blaustein, M.P. : How salt causes hypertension: The natriuretic hormone - Na/Ca exchange - hypertension hypothesis. Klinische Wochen-Schrift. 1985; 63 (Suppl. III), 82-85.

Borkowski, K.R. and Quinn, P. : Adrenaline and the development of spontaneous hypertension in rats. J. Auton. Pharmacol. 1985; 5, 89-100.

Burris, J.F., Waeber, B., Nussberger, J. and Brunner, H.R. : Enhanced acute antihypertensive effect of propranolol in the absence of circulating epinephrine in the rat. J. of Cardiovascular Pharmacol. 1984; 6, 697-700.

Campbell, W.B. and Jackson, E.K. : Modulation of adrenergic transmission by angiotensin in the perfused rat mesentery. Am. J. Physiol. 1979; 236, H211-H217.

Cheng, J.B. and Shibata, S. : Pressor response to 5-hydroxytryptamine norepinephrine and KCl in the perfused hindquarter preparation from spontaneously hypertensive rat. J. Pharmacol. Exp. Ther. 1980; 214, 488-495.

Cline, W.H., Jr. : Enhanced presynaptic angiotensin II receptor-mediated facilitation of in vivo vascular adrenergic neurotransmission in spontaneously hypertensive rats. Fred. Proc. 1982; 41, 1652.

Cline, W.H., Jr. : Enhanced effects of captopril and angiotensin II in vivo on vascular adrenergic neurotransmission in spontaneously hypertensive rats. Fed. Proc. 1983; 42, 892.

Clough, D.P., Hatton, R., Keddie, J.R. and Collis, M.G. : Hypotensive action of captopril in spontaneously hypertensive and normotensive rats. Interference with neurogenic vasoconstriction. Hypertension. 1982; 4, 764-772.

Cohen, M.L. and Berkowitz, B.A. : Decreased vascular relaxation in hypertension. J. Pharmacol. Exp. Ther 1976; 196, 396.

Colucci, W.S., Gimbrone, M.A., Jr., McLaughlin, M.K., Halpern, W. and Alexander, R.W. : Increased vascular catecholamine sensitivity and alpha -adrenergic receptor affinity in female and estrogen-treated male rats. Circ. Res. 1982; 50, 805-811.

Coote, J.H. and Sato, Y. : Reflex regulation of sympathetic activity in the spontaneously hypertensive rat. Circ. Res. 1977; 40, 571-577.

Day, M.D. and Moore, A.F. : Interaction of angiotensin II with noradrenaline and other spasmogens on rabbit isolated aortic strips. Arch. Int. Pharmacodyn. 1976; 219, 29-44.

Day, M.D., Owen, D.A.A. and Warren, P.R. : An adrenergic neuron blocking action of propranolol in isolated tissues. J. Pharm. Pharmacol. 1968; 20, 130s-134s.

Day, M.D., Roach, A.G. : Beta-adrenergic receptors in the central nervous system of the cat concerned with control of arterial blood pressure and heart rate. Nature New Biol. 1973; 242, 30-31.

De Jong, W., Lovenberg, W. and Sjoerdsma, A. : Increased plasma renin activity in the spontaneously hypertensive rat. Proceedings of the Society for Experimental Biology and Medicine. 1972; 139, 1213-1216.

Dengler, H. : Uber einen reninartigen Wirkstoff in Arterienextrakten. Achiv. fur Experimentelle Pathologie und Pharmakologie. 1956; 227, 481-487.

Desjardins-Giasson, S., Gutkowsa, J., Garcia, R. and Genest, J. : Renin substrate in rat mesenteric artery. Can.J. Physiol. Pharmacol. 1981; 59, 528-532.

Duckles, S.P. : Angiotensin II potentiates responses of the rabbit basilar artery to adrenergic nerve stimulation. Life Sci. 1980, 28, 635-640.

Dzau, V.J. : Vascular renin-angiotensin: A possible autocrine or paracrine system in control of vascular function. J. Cardiovasc. Pharmac. 1984; 6, S377-S382.

Eikenberg, D.C., Ekas, R.D. Jr. and Lokhandwale, M.R. : Presynaptic modulation of norepinephrine release by alpha -adrenoceptors and angiotensin II receptors in spontaneously hypertensive rats. Central Nervous System Mechanisms in Hypertension. 1981, 215-228, edited by J.P. Buckley and C.M. Ferrario, Raven Press, New York.

Eliash, S., Weinstock, M. : Role of adrenergic neurone blockade in the hypotensive action of propranolol. Br. J. Pharmacol. 1971; 43, 287-294.

Farnebo, L.O., Hamberger, B. : Influence of alpha-and beta- adrenoceptors on the release of noradrenaline from field stimulated atria and cerebral cortex slices. J. Pharm. Pharmacol. 1974; 26, 644-646.

Finch, L. and Leach, G.F.H. : Does the adrenal medulla contribute to the maintenance of experimental hypertension? Eur. J. Pharmacol. 1970; 11, 388-391.

Folkow, B. and Hallbeck, M. : Physiopathology of spontaneous hypertension in rats. In Hypertension Physiopathology and Treatment. 1977; 507-529, edited by Genest, J., Koiw, K. and Kuchel, O. New York: McGraw-Hill.

Folkow, B., Hallbeck, M., Lundgren, Y., Sivertsson, R., Weiss, L. : Importance of adaptive changes in vascular design for the establishment and maintenance of primary hypertension, as studied in man and in spontaneously hypertensive rats. In Spontaneous Hypertension. Its pathogenesis and complications. 1972; 103-114, edited by Okamoto, K. Tokyo, Igaku Shoin Ltd.

Frame, M.H., Hedqvist, P. : Evidence for prostaglandin mediated prejunctional control of renal sympathetic transmitter release and vascular tone. Br. J. Pharmacol. 1975; 54, 189-196.

Ganong, W.F. : Effects of sympathetic activity and ACTH on renin and aldosterone secretion. In Hypertension. 1972; 4-14, edited by Genest, J. and Koiw, E. Berlin, Springer-Verleg.

Garst, J.B., Koletsky, S., Wisenbaugh, P.E., Hadady, M. and Matthews, D. : Aterial wall renin and renal venous renin in the hypertensive rat. Clinical Science. 1979; 56, 41-46.

Garvey, H.L., Ram, N. : Centrally induced hypotensive effects of beta-adrenergic blocking drugs. Eur. J. Pharmacol 1975; 33, 283-294.

Glasser, S.R. : Influence of an adequate dietary protein on the immediate and latent effect of stilbesterol. Am. J. Physiol. 1954; 179, 421-428.

Goldblatt, H., Lynch, J., Hanzel, R.F., Summerville, W.W. : Studies on experimental hypertension. J. Exp. Med. 1934; 59, 347-379.

Gordon, R.D., Kuchel, O., Liddle, G.W. and Island, D.P. : Role of the sympathetic nervous system in regulating renin and aldosterone production in man. J. Clin. Invest. 1967; 46, 599-605.

Greenberg, S., Heitz , D.C. and Long, J.P. : Testosterone induced depression of adrenergic activity in the perfused canine hindlimb. Proc. Soc. Exp. Biol. Med. 1973; 142, 883-888.

Grobecker, H., Roizen, M.F., Weise, F., Saavedra, J.M. and Kopin, I.J. : Sympathoadrenal medullary activity in young hypertensive rats. Nature (Lond). 1975; 258, 267-268.

Grobecker, H., Saavedra, J.M. and Weise, V. : Biosynthetic enzyme activities and catecholamines in adrenal glands of genetic and experimental hypertensive animals. Clin. Sci. 1982; 63, 113s-116s.

Grollman, A.L. : Spontaneously hypertensive rat : Experimental analog of essential hypertension in the human being. In Spontaneous Hypertension : Its pathogenesis and complications. 1972; 239-242, edited by Okamoto, K. Tokyo, Igaku Shoin Ltd.

Hallback, M. and Folkow, B. : Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. Acta Physiol, Scand, 1974; 90, 684-698.

Harms, H.H. : Cardioselectivity of beta-adrenoreceptor blocking agents : in vitro studies with human and guinea-pig preparations. In : Beta-adrenoreceptor blocking agents. 1976; 311-315, edited by Sayena, P.R., Forsyth, R.P. Amsterdam, North Holland.

Hedqvist, P. and Moawad, A. : Presynaptic α-, and B-adrenoceptor mediated control of noradrenaline release in human oviduct. Acta Physiol. Scand. 1975; 95, 494-496.

Hill, R.W., Chester, J.E. and Wisenbaugh, P.E. : The effect of intravenous antirenin injection on chronic experimental and acute renin-induced hypertension in dogs. Laboratory Investigation 1970; 22, 404-410.

Hoffman, B.B., Lavin, T.N., Lefkowitz, R.J., Ruffolo, R.R. Jr. : Alpha-adrenergic receptor subtypes in rabbit uterus : Mediation of myometrial contraction and regulation by estrogens. J. Pharmacol. Exp. Ther. 1981; 219, 290-295.

Horovitz, Z.P., Editor : Angiotensin converting enzyme inhibitor. Baltimore : Urban and Schwarzenberg, 1981.

Iriuchijima, J. : Sympathetic discharge rate in spontaneously hypertensive rats. Jpn. Heart J. 1973; 14, 350-356.

Iso, T., Yamauchi H., Suda, H., Nakata, K., Nishimura, K., Iwao, J. : A new potent inhibitor of converting enzyme : (2R, 4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidine-carboxylic acid (SA-446). Jpn. J. Pharmacol. 1981; 31, 875-882.

Jackson, E.K. and Campbell, W.B. : Inhibition of neuronal noradrenaline uptake by angiotensin II in the rat mesentery. Can. J. Physiol. Pharmacol. 1979; 57, 1443-1447.

Judy, W.V., Wanatabe, A.M., Henry, D.P., Besch, H.R. Jr., Murphy, W.R. and Hockel, G.M. : Sympathetic nerve activity : Role in regulation of blood pressure in the spontaneously hypertensive rat. Circ. Res. 1976; 38 (Suppl II), 21-29.

Kamikawa, Y., Cline, W.H. Jr. and Su, C. : Diminished purinergic modulation of the vascular adrenergic neurotransmission in spontaneously hypertensive rats. Eur. J. Pharmacol. 1980; 66, 347-353.

Kawasaki, H., Cline, W.H., Jr. and Su, C. : Enhanced presynaptic beta-adrenoceptor-mediated modulation of vascular adrenergic neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 1982a; 223 (No. 3), 721-728.

Kawasaki, H., Cline, W.H., Jr. and Su, C. : Enhanced angiotensin-mediated facilitation of adrenergic neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 1982b; 221, 112-116.

Kawasaki, H., Cline, W.H., Jr. and Su, C. : Involvement of the vascular renin-angiotensin system in beta-adrenergic receptor-mediated facilitation of vascular neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 1984; 231 (No 1), 23-32.

Keeton, T.K. and Campbell, W.B. : The pharmacologic alteration of renin release. Pharmacol. Rev. 1980; 31, 81-227.

Kikta, D.C. and Fregly, M.J. : Effect of in vitro adminstration of captopril on vascular reactivity of rat aorta. Hypertension. 1982; 4, 118-124.

Koletsky, S., Rivera-Velez, J.M., Marsh, D.G. and Pritchard, W.H. : Relation of renal arterial pressure to activity of renin-angiotensin system in renal hypertension. Proc. Soc. Exp. Biol. Med. 1967; 125, 96-100.

Koletsky, S., Shook, P. and Rivera-Velez, J.M. : Lack of increased renin-angiotensin activity with spontaneous hypertension. Proc. Soc. Exp. Biol. Med. 1970; 134, 1187-1190.

Laffan, R.J., Goldberg, M.E., High, J.P., Schaeffer, T.R., Waugh, M.H., Rubin, B. : Antihypertensive activity in rats of SQ 14,225, an orally active inhibitor of angiotensin converting enzyme. J. Pharmacol. Exp. Ther. 1978; 204, 281-288.

Lands, A.M., Arnold,A., McAuliff, J.P., Luduena, F.O. and Brown, T.G. : Differentiation of receptor systems by sympathomimetic amines. Nature (Lond) 1967; 214, 597-598.

Langer, S.Z. : Presynaptic regulation of the release of catecholamines. Pharmacol. Rev. 1980; 32, 337-362.

Langer, S.Z., Enero, M.A., Adler-Graschinsky, E., Dubocovich, M.L. and Celuch, S.M. : Presynaptic regulatory mechanisms for noradrenaline release by nerve stimulation. In : Central action of drugs in blood pressure regulation. 1975; 133-150, edited by Davies, D.S. and Reid, J.L. Pitman, Tunbridge Wells.

Larsson, C. and Anggard, E. : Regional differences in the formation and metabolism of prostaglandins in rabbit kidney. Eur. J. Pharmacol. 1973; 21, 30-36.

Laverty, R. and Smirk, F.H. : Observations on the pathogenesis of spontaneous inherited hypertension and constricted renal-artery hypertension in rats. Circ. Res. 1961; 445-464.

Leonara, J. and Crane, M.G. : Effect of long term adminstration of medroxyprogesterone acetate and estrogens in the rat. Clin. Res. 1970; 19, 169.

Lew, G.M. : Change in blood pressure and norepinephrine concentration following adminstration of estrogen to genetically hypertensive and normotensive rats. Gen. Pharmacol. 1975; 6, 121-125.

Ljung, B., Jandhyala, B., Kjellstedt, A. : Angiotensin I converting enzyme activity in portal vein studied in normotensive rats and in models of primary and secondary hypertension. Acta Physiol. Scand. 1981; 111, 409-416.

Lokhandwala, M.F. and Eickenberg, D.C. : Presynaptic receptors and alterations in norepinephrine release in spontaneously hypertensive rats. Life Sci. 1983; 33, 1527-1542.

Majewski, H., Hedler, L. and Starke, K. : The noradreniline release rate in the anaesthetized rabbit: facilitation by adrenaline. Naunyn-Schmiedeberg's Arch. Pharmac. 1982a; 321, 20-27

Majewski, H., Rand, M.J., Tung, L.-H. : Activation of prejunctional B-adrenoceptors in rat atria by adrenalaine applied exogenously or released as a co-transmitter. Br. J. Pharmacol. 1981a; 73, 669-679.

Majewski, H., Tung, L.-H., Rand, M.J. : Adrenaline-induced hypertension in rats. J. Cardiovascular Pharmacol. 1981b, 179-185.

Majewski, H., Tung, L.-H., Rand, M.J. : Adrenaline activation of prejunctional B-adrenoceptors and hypertension. J. Cardiovascular Pharmacol. 1982b; 4, 99-105.

Malik, K.U. and Nasjletti, A. : Facilitation of adrenergic transmission by locally generated angiotensin II in rat mesenteric arteries. Circ. Res. 1976; 38, 26-30.

Matthews, R.J., Jr. : The effect of epinephrine, levarterenol and dl-isoproterenol on transmission in the superior cervical ganglion of the cat. J. Pharmacol. Exp. Ther. 1956; 116, 433-443.

McCarty, R. and Kopin, I.J. : Alterations in plasma catecholamines and behaviour during acute stress in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Life Sci. 1978; 22, 997-1006.

McCubbin, J.N. and Page, I.H. : Renal pressor system and neurogenic control of arterial pressure. Circ. Res. 1963; 12, 553-559.

McGregor, D.D. : The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. J. Physiol. (Lond). 1965; 177, 21-30.

McMurtry, J.P., Wright, G.L., Wrexler, B.C. : Spontaneous hypertension in cross-suckled rats. Science. 1981; 211, 1173-1175.

Meites, J. : Relation of food intake to growth depressing action of natural and artifical estrogen. Am. J. Physiol. 1949; 159, 281-289.

Michelakis, A.M., Caudle, J. and Liddle, G.W. : In vitro stimulation of renin production by epinephrine, norepinephrine and cyclic AMP. Proc. Soc. Exp. Biol. Med. 1969; 130, 748-753.

Michelakis, A.M. and McAllister, R.G. : Effect of chronic adrenergic receptor blockade on plasma renin activity in man. J. Chin. Endocrinol. Metab. 1972; 34, 386-394.

Minneman, K.P., Hedberg, A. and Molinoff, P.B. : Comparison of beta-adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Therap. 1979; 211, 502-508.

Misu, Y., Kuwahara, M., Kaiho, M. and Kubo, T. : Further characterisation of presynaptic B- adrenoceptors in guinea-pig pulmonary arteries. Eur. J. Pharmacol. 1983; 91, 287-290.

Molinoff, P.B., Axelrod, J. : Biochemistry of catecholamines. Annu. Rev. Biochem. 1971; 40, 465-500.

Morales-Aquilera, A., Vaughan-Williams, E.M. : The effects on cardiac muscle of B-receptor antagonist in relation to their activity as local anaesthetics. Br. J. Pharmacol. Chemother. 1965; 25, 332-338.

Mulvany, M.J. : Resistance vessel abnormalities in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 1984; 6, (Suppl. 4), S656-S665.

Musgrave, I.F., Bachmann, A.W., Gordon, R.D. : Increased plasma noradrenaline during adrenaline infusion in man. J. Hypertension. 1984; 2 (Suppl 3), 135-137.

Mylecharane, E.J. and Raper, C. : Prejunctional actions of some B-adrenoceptor antagonists in the vas deferens preparation of the guinea-pig. Br. J. Pharmacol. 1970; 39, 128-138.

Nagatsu, T., Ikuta, K., Numata, Y., Kato, T., Sano, M., Nagatsu, I., Umezawa, H., Matsuzaki, M. and Takeuchi, T. : Vascular and brain dopamine-B-hydroxylase activity in young spontaneously hypertensive rats. Science (Wash. DC). 1976; 191, 290-291.

Nakamura, K., Gerold, M., and Thoenen, H. : Experimental hypertension of the rat : reciprocal changes of norepinephrine turnover in heart and brain stem. Naunyn-Schmeideberg's Arch. Pharmakol, 1971; 268, 125-139.

Nakata, K., Nishimura, K., Takada, T., Ikuse, T., Yamauchi, H. and Iso, T. : Effects of an angiotensin-converting enzyme (ACE) inhibitor, SA 446, on tissue ACE activity in normotensive, spontaneously hypertensive, and renal hypertensive rats. J. Cardiovasc. Pharmacol. 1987; 9, 305- 310.

Nezu, M., Miura, Y., Adachi, M., Kimura, S., Toriyabe, S., Ischizuka, Y., Ohashi, H., Sugawara, T., Takahashi, M., Noshiro, T., Yoshinaga, K. : The role of epinephrine in essential hypertension. Jpn. Circ. J. 1983; 47, 1242-1246.

Nosaka, S., Wang, S.C. : Carotid sinus baroreceptor functions in the spontaneously hypertensive rat. Jpn. Circ. J. 1972; 34, 685-693.

Okamoto, K., Aoki, K. : Development of a strain of spontaneously hypertensive rats. Jap. Circ. J. 1963; 27, 282-293.

Okuno, T., Kondo, K., Konishi, K., Saruta, T., and Kato, E. : SQ 14,225 attenuates the vascular response to norepinephrine in the rat mesenteric arteries. Life Sci. 1979; 25, 1343-1350.

Palaic, D. and Khairallah, P.A. : Effect of angiotensin on uptake and release of norepinephrine by brain. Biochem. Pharmacol. 1967; 16, 2291-2298.

Pardo, E.G., Cato, J., Gijon, E., Alonso de Florida, F. : Influence of several adrenergic drugs on synaptic transmission through the superior cervical and the ciliary ganglion of the cat. J. Pharmacol. Exp. Ther. 1963; 139, 296-303.

Paton, W.D.M., Vizi, E.S. : The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br. J. Pharmacol. 1969; 35, 10-28.

Phelan, E.L. : The New Zealand strain of rats with genetic hypertension. New Zealand Medical Journal (special number). 1968; 67, 335-344.

Popper, C.W., Chieh, C.C. and Kopin, I.J. : Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization and after decapitation. J. Pharmacol. Exp. Ther. 1977; 202, 144-148

Rand, M.J., Law, M., Story, D.F., McCulloch, M.W. : Effects of B-adrenoreceptor blocking drugs on adrenergic transmission. Drugs. 1976; 11 (suppl 1), 134-143.

Rand, M.J., Majewski, H., McCulloch, M.W., Story, D.F. : An adrenaline-mediated positive feedback loop in sympathetic transmission and its possible role in hypertension 1979. In Presynaptic receptors. Langer, S.Z., Dubocovich, M.L. (eds), 263-269. Paris, Iuphar Satellite Meeting, Oxford. New York : Pergamon Press.

Reid, J.L., Lewis, P.J., Myers, M.G., Dollery, C.T. : Cardiovascular effects of intracerebroventricular d-, l-, and dl-propranolol in the conscious rabbit. J. Pharmacol. Exp. Ther. 1974; 188, 394-399.

Robert, J.M., Insel, P.A., Goldfien, R.D. and Goldfien, A. α-adrenoceptors but not β-adrenoceptors increase in rabbit uterus with estrogen. Nature 1977; 270, 624-625.

Rockson, S.G. Stone, R.A., Gunnells, J.C., Schanberg, S.M., Kirschner, N. and Robinson, R.R. : Plasma dopamine-B-hydroxylase activity in oral contraceptive hypertension. Circulation. 1975; 51, 916-923.

Rosenthal, J., Boucher, R., Rojo-Ortega, J.M. and Genest, J. : Renin activity in aortic tissue of rats. Can. J. Pharmacol. 1969; 47, 53-56.

Roth, R.A. and Wallace, K.B. : Disposition of biogenic amines and angiotensin I by lungs of spontaneously hypertensive rats. Am. J. Physiol. 1980; 239, H736-H741.

Schaechtelin, G., Regoli, O and Gross, F. : Quantitative assay and disappearance rate of circulating renin. Am. J. Physiol. 1964; 206, 1361-1364.

Sen, S. Bravo, E.L. and Bumbus, F.M. : Isolation of a hypertension producing compound from normal human urine. Circ. Res. 1977; 40 (suppl 1), I5-I10.

Sokabe, H. : Renin activity of the kidney in the spontaneous hypertensive rat. Jpn. J. Physiol. 1966; 16, 380-388

Starke, K. : Action of angiotensin on uptake, release and metabolism of ^{14}C-noradrenaline by isolated rabbit hearts. Eur. J. Pharmacol. 1971; 14, 112-123.

Starke. K. : Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 1977; 77, 1-124.

Starke, K., Gorlitz, B.O., Montel, H., Schumann, H.J. : Local alpha-adrenoceptor mediated feedback inhibition of catecholamine release from the adrenal medulla. Experienta. 1974; 30, 1170-1171.

Starke, K., Eundo, T., Taube, H.D. : Relative pre- and post-synaptic potencies of alpha-adrenoceptor agonists in the rabbit pulmonary artery. Naunyn Schmiedebergs Arch. Phamacol. 1975; 291, 55.78.

Starke, K. and Langer, S.Z. : A note on terminology for presynaptic receptors. In Advances in the biosciences : presynaptic receptors. Vol. 18, 1-3. Editors, Langer, S.Z., Starke, K. and Dubocovich, M.L. Pergamon Press, Oxford.

Stevens, M.J., Ritinghausen R.E., Medcalf, R.L. and Moulds, R.F.W. : Prejunctional B-adrenoceptors in human digital arteries. Eur. J. Pharmacol. 1982; 83, 263-270.

Stjarne, L. : Adrenoreceptor mediated positive and negative feedback control of noradrenaline secretion from human vasoconstrictor nerves. Acta. Physiol. Scand. 1975; 95, 18A-19A.

Stjarne, L. and Brundin, J. : Dual adrenoceptor-mediated control of noradrenaline secretion from human vasoconstrictor nerves : Facilitation by B-receptors and inhibition by alpha-receptors. Acta. Physiol. Scand. 1975; 94, 139-141.

Stjarne, L. and Brundin, J. : B_2-adrenoceptors facilitating noradrenaline secretion from human vasoconstrictor nerves. Acta. Physiol. Scand. 1976; 97, 267-269.

Sweet, C.S. Blain, E.H. : In cardiovascular pharmacology. 1984; edited by Antonaccio, M.J. 2nd ed. New York, Raven Press.

Tanigawa, H. Allison, D.J. and Assaykeen, T.A. : Comparison of the effects of various carecholamines on plasma renin activity alone and in the presence of adreneregic blocking agents. In Hypertension 1972; 37-44. Edited by Genest, J. (ION) and Koiw, E. Berlin. Springer-Verlag.

Teitelman, G., Ross, R.A., John, T.H. and Reis, D.J. : Differences in utero in activitites of catecholamine biosynthetic enzymes in adrenals of spontaneously hypertensive rats. Clin. Sci. 1981; 61, 227s-230s.

Thurston, H., Swales, J.D. : Action of angiotensin antagonists and antiserum upon the pressor response to renin further evidence for the local generation of angiotensin II. Cli. Sci. Molec. Med. 1974; 46, 273-276.

Thurston, H., Swales, J.D. : Blood pressure response of nephrectomized hypertensive rats to converting enzyme inhibitor : evidence for persistence vascular renin activity. Cli. Sci. Molec. Med. 1977; 52, 299-304.

Trinner, L., Vulliemoz, M., Verosky, M. and Manger, W.N. : Cyclic adrenosine monophosphate and vascular reactivity in spontaneously hypertensive rats. Biochem. Pharmacol. 1975; 24, 743-745.

Vander, A.J. : Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs. Am. J. Physiol. 1965; 209, 659-662.

Velletri, P., Bean, B.L. : The effects of captopril on rat aortic angiotensin-converting enzyme. J. Cardiovasc. Pharmacol. 1982; 4, 315-325.

Vincent, H.H., Man in't Veld, A.J., Boomsma, F., Dertx, F. and Schalekamp, A.D.H. : Compound ICI 118,551, a B_2-adrenoceptor antagonist, lowers blood pressure. J. Hypertension. 1985; 3 (suppl 3), S247-S249.

Vincent, H.H., Boomsma, F., Man in't Veld, A.J., Schalekemp, A.D.M. : Beta-receptor stimulation by adrenaline elevates plasma noradrenaline and enhances the pressor responses to cold exposure and isometric exercise. J. Hypertension. 1983; 1 (suppl 2), 74-76.

Vincent, M., Dupong, J. and Sassard, J. : P lasma renin activity as a function of age in two new strains of spontaneously hypertensive and normotensive rats. Cli. Sci. Molec. Med. 1976; 50, 103-107.

Vlachakis, N.D., Alexander, N. and Maronde, R.H. : Increased plasma normetanephrine in spontaneously hypertensive rats. Cli. Exp. Hypertension. 1980; 2, 309-319.

Wagermark, J., Ungerstedt, U. and Ljungqvist, A. : Sympathetic innervation of the juxtagomerular cells of the kidney. Circ. Res. 1968; 22, 149-153.

Westfall, T.C. : Local regulation of adrenergic neurotranmission. Physiol. Rev. 1977; 57, 659-728.

Westfall, T.C. and Brasted, M. : Specificity of blockade of the nicotine-induced release of ^{3}H-norepinephrine from adrenergic neurons of the guinea-pig heart by various pharmacological agents. J. Pharmacol. Exp. Ther. 1974; 189, 659-664.

Westfall, T.C., Peach, M.J. and Tittermar, V. : Enhancement of the electrically induced release of norepinephrine from the rat portal vein. Mediation by B_2-adrenocedptors. Eur. J. Pharmacol. 1979; 58, 67-74.

Wocial, B., Chodakowska, J., Zukowska, _Brojec, Z., Rutczynski, M. and Boratynski, W. : Tissue catecholamine concentrations in spontaneously hypertensive rats. Acta. Physiol. Pol. 1977; 28, 303-3 12.

Yamori, Y. : Contribution of cardiovascular factors to the development of hypertension in spontaneously hypertensive rats. Jpn. Heart. J. 1974; 15, 194-196.

Yamori, Y., Okamoto,, K. : Hypothalamic tonic regulation of blood pressure in spontaneously hypertensive rats. Jap. Circ. J. 1969; 33, 509-519.

Yamori, Y., de Yong, W., Yamabe, H., Lovenberg, W., Sjaerdsma, A. : Effects of L-dopa and inhibitors of decarboxylase and monamine oxidase on brain noradrenaline levels and blood pressure in spontaneously hypertensive rats. J. Pharm. Pharmacol. 1972; 24, 690-695.

Addendum

Altura, B.M. : Sex and estrogens and responsiveness of terminal arteriols to neurohypophyseal hormones and catecholamines . J. Pharmacol. Exp. Ther. 1975; 193, 403-412.

Dahlof, C., Ljung, B., Ablad B. : Increased noradrenaline release in rat portal vein during sympathetic nerve stimulation due to activation of presynaptic B-adrenoreceptors by noradrenaline and adrenaline. Eur. J. Pharmacol. 1978; 50, 75-78.

DeChamplain, J. : Experimental aspects of the relationships between the autonomic nervous systema and catecholamines in hypertension. In Hypertension, 1977; 76-92. eds, Genest, J., Koiw, E. and Kuchel, O. New York, McGraw Hill.

Lew, G.M. : Effects of mestranol on blood pressure and norepinephrine in young normotensive and genetically hypertensive rats. Gen. Pharmacol. 1978; 9, 163-166.

Lew, G.M. : Age differences in blood pressure and morepinephrine concentration in normotensive rats after higher doses of estrogens. Gen. Pharmacol. 1982; 13, 75-78.

McGregor, D.D. and Smirk, F.H. : Vascular responses to 5-hydroxytryptamine in genetic and renal hypertensive rats. Am. J. Physiol. 1970; 219, 687-690.

Appendix

Draper, A.J., Meghji, S.A., Redfern, P.H. : Facilitatory pre-synaptic receptors in the isolated perfused rat mesentery. Proceedings of the British Pharmacological Society. April, 1986. P181.

P.181

FACILITATORY PRE-SYNAPTIC RECEPTORS IN THE ISOLATED PERFUSED RAT MESENTERY

A.J. Draper, S.A. Meghji and P.H. Redfern, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

It is generally accepted that presynaptic receptors modulate neurotransmitter release from sympathetic nerve endings (Starke, 1981). Various neurohumoral agents (Angiotensin II and beta-adrenoceptor agonists) have been shown to facilitate this release. Demonstration of the existence of a complete renin-angiotensin system in the rat mesenteric vascular wall (Desjardins-Giasson et al, 1981) raises the possibility of an involvement of this system in the isoprenaline (Iso)-induced facilitation of adrenergic neurotransmission (Kawasaki et al, 1984). Male and female Japanese Spontaneously Hypertensive rats (SHR, m and SHR, f, respectively) and New Zealand Hypertensive rats (NZH, f) 190-260g were used. The isolated perfused mesentery was set up as described by McGregor (McGregor, 1964) and increases in perfusion pressure in response to periarterial nerve stimulation (PNS) (80V, 1msec, 30Hz for 10sec) were recorded in the presence of a range of concentration of Iso. It was found that in all three groups Iso caused a significant dose-dependent potentiation of the pressor response to PNS. This potentiation was blocked by ICI 118,551 but not by atenolol. Addition of angiotensin II (AII), 10ng/ml, to the perfusate significantly potentiated the pressor response to PNS. This potentiation was attenuated by [Sar1-Ile8]angiotensin II (SAR), an AII antagonist. The SAR itself markedly attenuated the ISO-induced potentiation of the pressor response to PNS; however, ICI 118,551 did not affect the AII potentiation of the pressor response.

TABLE 1

	% Perfusion pressure in response to PNS (control 100%)				
	ISO ($5x10^{-8}$M)	ISO + atenolol (10^{-7}M)	ISO + ICI118,551 ($5x10^{-7}$M)	AII (10ng/ml)	ISO + SAR (200ng/ml)
SHR, m	150+/-4	207+/-11	95+/-5	158+/-7	104+/-4
SHR, f	140+/-5	142+/-5	99+/-2	150+/-8	103+/-5
NZH, f	179+/-3	180+/-4	97+/-2	153+/-6	101+/-10

Values are Mean +/- s.e.m. n=4 All values are statistically significant ($p<0.01$).

The results suggest that the potentiation of the effect of PNS by Iso involves activation of presynaptic beta 2 adrenoceptors and that this in turn may require activation of the renin-angiotensin system.

Desjardins-Giasson, S.; Gutowska, J.; Garcia, R.; and Genest, J.: Can. J. Physiol. Pharmacol. 59:528-532, 1981.

Kawasaki, H.; Cline, W.H., Jnr.; Su, C.; J. Pharmacol. Exp. Ther. 231:23-32, 1984.

McGregor, D.D.: J. Physiol. (London) 177:21-30, 1965.

Starke, K.: Ann. Rev. Pharmacol. 21:7-30, 1981.